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**From:** Mowatt, Michael (NIH/NIAID) [E] [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=CB1EF7E2E54B4164AE34814574BDA638-MMOWATT]  
**Sent:** 11/7/2017 1:45:33 PM  
**To:** Rohrbaugh, Mark (NIH/OD) [E] [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=591ab6b2424b4b8997082718cbb29fab-rohrbaum]  
**CC:** Frisbie, Suzanne (NIH/NIAID) [E] [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=c402740ceaad4d4f97a8c28f16fbb349-frisbies]; Mowatt, Michael (NIH/NIAID) [E] [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=cb1ef7e2e54b4164ae34814574bda638-mmowatt]  
**Subject:** FW: Proposed grant of an exclusive license to Zika Vaccine

Mark,

Suzanne has posed a few questions, which I've embellished. We're interested in your perspectives.

b5

Thanks,

Mike

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**From:** Frisbie, Suzanne (NIH/NIAID) [E]  
**Sent:** Monday, November 6, 2017 6:36 PM  
**To:** Mowatt, Michael (NIH/NIAID) [E] <mmowatt@niaid.nih.gov>  
**Subject:** Re: Proposed grant of an exclusive license to Zika Vaccine

Hi Mike,

I have no edits to the draft, just a few thoughts. I am wondering if

b5

b5

Suzanne

Sent from my iPhone

On Nov 6, 2017, at 4:57 PM, Mowatt, Michael (NIH/NIAID) [E] <mmowatt@niaid.nih.gov> wrote:

See proposed draft (attached).

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**From:** Stover, Kathy (NIH/NIAID) [E]  
**Sent:** Friday, November 3, 2017 10:35 AM  
**To:** Rohrbaugh, Mark (NIH/OD) [E] <rohrbaum@od.nih.gov>; Petrik, Amy (NIH/NIAID) [E]

REL0000023990

<amy.petrik@nih.gov>; Mowatt, Michael (NIH/NIAID) [E] <mmowatt@niaid.nih.gov>  
**Cc:** Salata, Carol (NIH/NIAID) [E] <csalata@niaid.nih.gov>; Feliccia, Vincent (NIH/NIAID) [E]  
<vfeliccia@niaid.nih.gov>; Greco, Natalie (NIH/NIAID) [C] <natalie.greco@nih.gov>; Frisbie, Suzanne  
(NIH/NIAID) [E] <suzanne.frisbie@nih.gov>  
**Subject:** RE: Proposed grant of an exclusive license to Zika Vaccine

Hi all,

In talking it over here at NIAID OCGR, we think

b5

b5

Best,  
Kathy

Kathy Stover  
Branch Chief  
News and Science Writing Branch  
National Institute of Allergy and Infectious Diseases (NIAID)  
Office of Communications and Government Relations  
National Institutes of Health/HHS  
31 Center Drive, Room 7A17E  
Bethesda, MD 20892  
Phone: (301) 496-8864  
E-mail: [kstover@nih.gov](mailto:kstover@nih.gov)  
NIAID Media Line: (301) 402-1663

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**From:** Rohrbaugh, Mark (NIH/OD) [E]  
**Sent:** Friday, November 03, 2017 9:59 AM  
**To:** Petrik, Amy (NIH/NIAID) [E] <amy.petrik@nih.gov>; Mowatt, Michael (NIH/NIAID) [E]  
<mmowatt@niaid.nih.gov>  
**Cc:** Salata, Carol (NIH/NIAID) [E] <csalata@niaid.nih.gov>; Feliccia, Vincent (NIH/NIAID) [E]  
<vfeliccia@niaid.nih.gov>; Greco, Natalie (NIH/NIAID) [C] <natalie.greco@nih.gov>; Frisbie, Suzanne  
(NIH/NIAID) [E] <suzanne.frisbie@nih.gov>; Stover, Kathy (NIH/NIAID) [E] <kathy.stover@nih.gov>  
**Subject:** RE: Proposed grant of an exclusive license to Zika Vaccine

b5

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**From:** Petrik, Amy (NIH/NIAID) [E]  
**Sent:** Friday, November 03, 2017 8:31 AM  
**To:** Mowatt, Michael (NIH/NIAID) [E] <mmowatt@niaid.nih.gov>  
**Cc:** Salata, Carol (NIH/NIAID) [E] <csalata@niaid.nih.gov>; Feliccia, Vincent (NIH/NIAID) [E]  
<vfeliccia@niaid.nih.gov>; Greco, Natalie (NIH/NIAID) [C] <natalie.greco@nih.gov>; Frisbie, Suzanne  
(NIH/NIAID) [E] <suzanne.frisbie@nih.gov>; Stover, Kathy (NIH/NIAID) [E] <kathy.stover@nih.gov>;  
Rohrbaugh, Mark (NIH/OD) [E] <rohrbaum@od.nih.gov>  
**Subject:** FW: Proposed grant of an exclusive license to Zika Vaccine

Hi Mike,

REL0000023990

Below is the message from KEI.

Thanks,  
Amy

**From:** Kim Treanor [<mailto:kim.treanor@keionline.org>]  
**Sent:** Wednesday, October 25, 2017 2:53 PM  
**To:** Petrik, Amy (NIH/NIAID) [E] <[amy.petrik@nih.gov](mailto:amy.petrik@nih.gov)>  
**Subject:** Proposed grant of an exclusive license to Zika Vaccine

Dear Dr. Petrik,

I am writing in regards to the proposed grant of an exclusive patent license of a DNA-based vaccine for prevention of Zika virus infection to PaxVax Inc, as referenced in 82 FR 47537. As a part of this licensing agreement or separately from it, if the exclusive license is granted, will the NIAID or another division of the NIH also provide PaxVax with grants or financial support to conduct clinical trials on this vaccine candidate? PaxVax reports on their website that they have a Zika vaccine candidate in the pipeline which they are working on with the CDC. Do you know if this vaccine candidate has received any financial support from NIAID or another division of the NIH?

Thank you for your assistance.

Best regards,  
Kim

--

Kim Treanor  
Knowledge Ecology International  
[kim.treanor@keionline.org](mailto:kim.treanor@keionline.org)  
tel.: +1.202.332.2670

<Response to KTreanor DRAFT 171106.docx>

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**From:** Wolinetz, Carrie (NIH/OD) [E] [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=1C655040D47346C7B04D7BC11A403ECB-WOLINETZCD]  
**Sent:** 7/17/2019 3:35:58 PM  
**To:** Jorgenson, Lyric (NIH/OD) [E] [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=3bbde7d361374981a4d336b6eeb17521-jorgensonla]; Rohrbaugh, Mark (NIH/OD) [E] [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=591ab6b2424b4b8997082718cbb29fab-rohrbaum]  
**CC:** Plude, Denise (NIH/OD) [E] [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=91f83d681d984eaa8fe3de287aebfa01-pludedede]; Ampey, Bryan (NIH/OD) [E] [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=9672b522d0b34f3792e2934dac636a57-ampeybc]; Bayha, Ryan (NIH/OD) [E] [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=5d5a4353cd514322a8598dbb1751ee79-bayhar]  
**Subject:** Re: IMPORTANT FW: WF 384359 - Direct Reply due 7/18

I think b5

----- Original Message -----

**From:** "Jorgenson, Lyric (NIH/OD) [E]" <lyric.jorgenson@nih.gov>  
**Date:** Wed, Jul 17, 2019, 11:34 AM  
**To:** "Rohrbaugh, Mark (NIH/OD) [E]" <rohrbaum@od.nih.gov>, "Wolinetz, Carrie (NIH/OD) [E]" <carrie.wolinetz@nih.gov>  
**CC:** "Plude, Denise (NIH/OD) [E]" <pludedede@mail.nih.gov>, "Ampey, Bryan (NIH/OD) [E]" <bryan.ampey@nih.gov>, "Bayha, Ryan (NIH/OD) [E]" <bayhar@od.nih.gov>  
**Subject:** RE: IMPORTANT FW: WF 384359 - Direct Reply due 7/18

I think that this looks great. I am not sure who should sign – b5  
b5

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**From:** Rohrbaugh, Mark (NIH/OD) [E] <rohrbaum@od.nih.gov>  
**Sent:** Tuesday, July 16, 2019 7:03 PM  
**To:** Jorgenson, Lyric (NIH/OD) [E] <lyric.jorgenson@nih.gov>  
**Cc:** Plude, Denise (NIH/OD) [E] <pludedede@mail.nih.gov>; Ampey, Bryan (NIH/OD) [E] <bryan.ampey@nih.gov>; Bayha, Ryan (NIH/OD) [E] <bayhar@od.nih.gov>  
**Subject:** IMPORTANT FW: WF 384359 - Direct Reply due 7/18

b5 They have joined KEI  
on a few objections to proposed IRP patent licenses but nothing else.

See attached draft response. b5

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**From:** Jorgenson, Lyric (NIH/OD) [E]  
**Sent:** Friday, July 12, 2019 10:53 AM  
**To:** Plude, Denise (NIH/OD) [E] <pludedede@mail.nih.gov>  
**Cc:** Bayha, Ryan (NIH/OD) [E] <bayhar@od.nih.gov>; Ampey, Bryan (NIH/OD) [E] <bryan.ampey@nih.gov>; Koniges, Ursula (NIH/OD) [E] <ursula.koniges@nih.gov>; Fennington, Kelly (NIH/OD) [E] <fenningk@od.nih.gov>; Wertz, Jennifer (NIH/OD) [E] <wertzj@od.nih.gov>  
**Subject:** Re: WF 384359 - Direct Reply due 7/18

b5



Lyric Jorgenson, PhD  
Deputy Director, Office of Science Policy  
National Institutes of Health  
301.496.6837

On Jul 12, 2019, at 7:39 AM, Plude, Denise (NIH/OD) [E] <[pludedede@mail.nih.gov](mailto:pludedede@mail.nih.gov)> wrote:

b5

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**From:** Bayha, Ryan (NIH/OD) [E]  
**Sent:** Tuesday, July 09, 2019 6:40 PM  
**To:** Ampey, Bryan (NIH/OD) [E] <[bryan.ampey@nih.gov](mailto:bryan.ampey@nih.gov)>; Koniges, Ursula (NIH/OD) [E] <[ursula.koniges@nih.gov](mailto:ursula.koniges@nih.gov)>; Plude, Denise (NIH/OD) [E] <[pludedede@mail.nih.gov](mailto:pludedede@mail.nih.gov)>; Fennington, Kelly (NIH/OD) [E] <[fenningk@od.nih.gov](mailto:fenningk@od.nih.gov)>; Jorgenson, Lyric (NIH/OD) [E] <[lyric.jorgenson@nih.gov](mailto:lyric.jorgenson@nih.gov)>  
**Cc:** Wertz, Jennifer (NIH/OD) [E] <[wertzj@od.nih.gov](mailto:wertzj@od.nih.gov)>  
**Subject:** RE: WF 384359 - Direct Reply due 7/18

Since this is direct reply,

b5

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**From:** Ampey, Bryan (NIH/OD) [E] <[bryan.ampey@nih.gov](mailto:bryan.ampey@nih.gov)>  
**Sent:** Tuesday, July 9, 2019 3:30 PM  
**To:** Koniges, Ursula (NIH/OD) [E] <[ursula.koniges@nih.gov](mailto:ursula.koniges@nih.gov)>; Plude, Denise (NIH/OD) [E] <[pludedede@mail.nih.gov](mailto:pludedede@mail.nih.gov)>; Bayha, Ryan (NIH/OD) [E] <[bayhar@od.nih.gov](mailto:bayhar@od.nih.gov)>; Fennington, Kelly (NIH/OD) [E] <[fenningk@od.nih.gov](mailto:fenningk@od.nih.gov)>; Jorgenson, Lyric (NIH/OD) [E] <[lyric.jorgenson@nih.gov](mailto:lyric.jorgenson@nih.gov)>  
**Cc:** Wertz, Jennifer (NIH/OD) [E] <[wertzj@od.nih.gov](mailto:wertzj@od.nih.gov)>  
**Subject:** RE: WF 384359 - Direct Reply due 7/18

Mark passed this along earlier too for awareness. This is the same group requesting a direct reply

Here is the link: <https://www.statnews.com/2019/07/09/dc-diagnosis-drug-prices-tv-ads/>

## Drug pricing advocates put NIH in the hot seat

The drug industry foe Patients For Affordable Drugs has a new report out this morning arguing that taxpayers have contributed at least \$300 million toward the development of a gene therapy to cure sickle cell disease — and the group says that's reason enough for the NIH to demand the treatment be reasonably priced.

“Given the \$1 to \$2 million price range of recent gene therapies, we are concerned that a sickle cell cure will be brought to market at a price that is unaffordable for patients and for the taxpayers who supported its development,” the group writes. “The NIH should use all levers in its power to ensure the final price accounts for public investment.”

The group has a number of suggestions to NIH on how to establish pricing guardrails, including requiring that the drug maker price the drug at no more than the average of comparable OECD nations.

This isn't the first time drug pricing advocates have railed against NIH licensing out government-developed drugs without restricting what drug makers can charge, but those complaints so far have fallen on deaf ears.

An NIH spokesperson declined to comment on P4AD's pricing concerns and emphasized that NIH does not have a role in setting prices. The spokesperson also disputed P4AD's argument that \$300 million went to the development of this one particular therapy, because the NIH studies were foundational research studies. "You can't take foundational studies and apply them to one product," the spokesperson said.

Bryan Ampey, Ph.D.  
Health Science Policy Analyst  
Office of Science Policy, Office of the Director  
National Institutes of Health  
301-451-6346

<image001.png>

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**From:** Koniges, Ursula (NIH/OD) [E]  
**Sent:** Tuesday, July 09, 2019 3:27 PM  
**To:** Plude, Denise (NIH/OD) [E] <pludedede@mail.nih.gov>; Ampey, Bryan (NIH/OD) [E] <bryan.ampey@nih.gov>; Bayha, Ryan (NIH/OD) [E] <bayhar@od.nih.gov>; Fennington, Kelly (NIH/OD) [E] <fenningk@od.nih.gov>; Jorgenson, Lyric (NIH/OD) [E] <lyric.jorgenson@nih.gov>  
**Cc:** Wertz, Jennifer (NIH/OD) [E] <wertzj@od.nih.gov>  
**Subject:** RE: WF 384359 - Direct Reply due 7/18

Please assign this to Mark. Thanks Denise!

---

**From:** Plude, Denise (NIH/OD) [E] <pludedede@mail.nih.gov>  
**Sent:** Tuesday, July 09, 2019 3:26 PM  
**To:** Ampey, Bryan (NIH/OD) [E] <bryan.ampey@nih.gov>; Bayha, Ryan (NIH/OD) [E] <bayhar@od.nih.gov>; Fennington, Kelly (NIH/OD) [E] <fenningk@od.nih.gov>; Jorgenson, Lyric (NIH/OD) [E] <lyric.jorgenson@nih.gov>; Koniges, Ursula (NIH/OD) [E] <ursula.koniges@nih.gov>  
**Cc:** Wertz, Jennifer (NIH/OD) [E] <wertzj@od.nih.gov>  
**Subject:** WF 384359 - Direct Reply due 7/18

### **Work Folder Information**

**Work Folder:** WF 384359

**Process:** Direct Reply

**Program Analyst:** Whitfield, Michelle D. (NIH/OD) [E]

**Due Date:** July 18, 2019

**WF Subject:** Writer urges NIH to review policies under which taxpayer-funded discoveries are licensed to private corporations that then price the drug out of reach for patients and our health system.

**IC:** od\_osp

**From:** Mitchell, David

**To:** Collins, Francis

**Remarks:** Assigned to OSP for direct reply with clearance by 7/18/19. Please prepare a draft response and submit to ES by cob 7/18/19 for clearance. Once the response has been cleared, ES will return to OSP for mailing. Also, if OSP feels this should be a Dir Sig or DepD Sig, please let me know. Thank you.

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**From:** Jambou, Robert (NIH/OD) [E] [/O=NIH/OU=NIHEXCHANGE/CN=OD/CN=JAMBOUR]  
**Sent:** 7/19/2016 9:23:14 PM  
**To:** Rohrbaugh, Mark (NIH/OD) [E] [/O=NIH/OU=NIHEXCHANGE/cn=OD/cn=ROHRBAUM]  
**Subject:** RE: FOIA Request - 45260\_Goldman re: Union for Affordable Cancer Treatment (UACT)

Hi Mark,

Are you busy now? Or would some other time be better?

*Bob J.*

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**From:** Rohrbaugh, Mark (NIH/OD) [E]  
**Sent:** Tuesday, July 19, 2016 5:00 PM  
**To:** Jambou, Robert (NIH/OD) [E] <JambouR@OD.NIH.GOV>  
**Subject:** RE: FOIA Request - 45260\_Goldman re: Union for Affordable Cancer Treatment (UACT)

Bob:

I thought I knew how to find the pst file, but I cannot. I have the emails in a separate folder saved in Outlook.

If it is easier to talk me through it, let me know when you are available.

Thanks,  
Mark

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**From:** Jambou, Robert (NIH/OD) [E]  
**Sent:** Friday, July 15, 2016 5:31 PM  
**To:** Rohrbaugh, Mark (NIH/OD) [E] <RohrBauM@OD.NIH.GOV>  
**Cc:** Wolinetz, Carrie (NIH/OD) [E] <carrie.wolinetz@nih.gov>; Carr, Sarah (NIH/OD) [E] <CarrS@OD.NIH.GOV>; Dodson, Sara (NIH/OD) [E] <sara.dodson@nih.gov>  
**Subject:** RE: FOIA Request - 45260\_Goldman re: Union for Affordable Cancer Treatment (UACT)

Hi Mark, thanks for the feedback.

The best way to get the responsive records to me is to create a separate PST file on your PC or Home drive and copy the responsive e-mails there.

Once the e-mails are copied over,  
"Close" the PST file in the gray Left Navigation panel of Outlook.  
Then you must shut down Outlook completely.  
Open the location of the PST file and copy/move that file to: \\odapps4\oba-shared\FOIA\UACT\

The other alternative is to send your e-mails as attachments (aka "Outlook Items") in an e-mail addressed to me— if you have a lot of attachments, you will need to send several; I think the max e-mail size is around 50 MB.

Let me know if you need some help with this.

Best,

*Bob J.*

REL0000023992

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**From:** Rohrbaugh, Mark (NIH/OD) [E]  
**Sent:** Thursday, July 14, 2016 4:32 PM  
**To:** Jambou, Robert (NIH/OD) [E] <JambouR@OD.NIH.GOV>  
**Cc:** Wolinetz, Carrie (NIH/OD) [E] <carrie.wolinetz@nih.gov>; Carr, Sarah (NIH/OD) [E] <CarrS@OD.NIH.GOV>; Dodson, Sara (NIH/OD) [E] <sara.dodson@nih.gov>; Volkov, Marina (NIH/OD) [E] <mvolkov@od.nih.gov>; Fennington, Kelly (NIH/OD) [E] <FenningK@OD.NIH.GOV>  
**Subject:** RE: FOIA Request - 45260\_Goldman re: Union for Affordable Cancer Treatment (UACT)

I have over 100 emails that mention Xtandi, most of them internal and predecisional or questions about status of this or that. What is the best way to get them to you?

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**From:** Jambou, Robert (NIH/OD) [E]  
**Sent:** Thursday, July 07, 2016 12:47 PM  
**To:** Rohrbaugh, Mark (NIH/OD) [E] <RohrBauM@OD.NIH.GOV>; Wolinetz, Carrie (NIH/OD) [E] <carrie.wolinetz@nih.gov>; Carr, Sarah (NIH/OD) [E] <CarrS@OD.NIH.GOV>; Dodson, Sara (NIH/OD) [E] <sara.dodson@nih.gov>; Volkov, Marina (NIH/OD) [E] <mvolkov@od.nih.gov>; Fennington, Kelly (NIH/OD) [E] <FenningK@OD.NIH.GOV>  
**Subject:** FOIA Request - 45260\_Goldman re: Union for Affordable Cancer Treatment (UACT)

Hi all,

Please see the attached request. I am notifying you of a FOAI request received today 7/7/2016.  
The requester is Andrew Goldman, Counsel for Knowledge Ecology International.  
The scope of the response is January 14, 2016 through July 7, 2016 (the date the net is cast).

Please do not delete any e-mails or other records responsive to this request.  
Please begin your search ASAP as this request was received by NIH on June 29, 2016 and ideally should be completed within 20 working days.

When you are ready to provide responsive records, please notify me that I can assist in making this as efficient as possible.

Thanks to all...

**Bob J.**

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**From:** Hammersla, Ann (NIH/OD) [E] [/O=NIH/OU=NIHEXCHANGE/CN=RECIPIENTS/CN=HAMMERSLAA]  
**Sent:** 5/4/2017 5:21:28 PM  
**To:** Rohrbaugh, Mark (NIH/OD) [E] [/O=NIH/OU=NIHEXCHANGE/cn=OD/cn=ROHRBAUM]  
**Subject:** FW: Final Final Draft  
**Attachments:** Response 05042017 KEI request REV4 ahmr.docx

Mark: I didn't catch your sentence below, I added b5 to this 4<sup>th</sup> draft . Ann

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**From:** Hammersla, Ann (NIH/OD) [E]  
**Sent:** Thursday, May 04, 2017 1:10 PM  
**To:** Rohrbaugh, Mark (NIH/OD) [E] <RohrBauM@OD.NIH.GOV>  
**Subject:** RE: Final Final Draft

How is this for the final? Ann

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**From:** Rohrbaugh, Mark (NIH/OD) [E]  
**Sent:** Thursday, May 04, 2017 1:01 PM  
**To:** Hammersla, Ann (NIH/OD) [E] <[hammerslaa@mail.nih.gov](mailto:hammerslaa@mail.nih.gov)>  
**Subject:** RE: Final Draft

# b5

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**From:** Hammersla, Ann (NIH/OD) [E]  
**Sent:** Thursday, May 04, 2017 12:46 PM  
**To:** Rohrbaugh, Mark (NIH/OD) [E] <[RohrBauM@OD.NIH.GOV](mailto:RohrBauM@OD.NIH.GOV)>  
**Subject:** Final Draft

Mark: Attached is the final draft of the KEI response. I made a couple of small edits to the last draft. Ok? Ann

**b5**

**b5**



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**From:** Hammersla, Ann (NIH/OD) [E] [/O=NIH/OU=NIHEXCHANGE/CN=RECIPIENTS/CN=HAMMERSLAA]  
**Sent:** 5/1/2017 7:13:48 PM  
**To:** Rohrbaugh, Mark (NIH/OD) [E] [/O=NIH/OU=NIHEXCHANGE/cn=OD/cn=ROHRBAUM]  
**Subject:** FW: Response Creation - DUE by COB Friday April 30th (WF 357204)  
**Attachments:** Response KEI 04282017 05012017request.docx

**From:** Hammersla, Ann (NIH/OD) [E]  
**Sent:** Monday, May 01, 2017 11:20 AM  
**To:** Brandy, Aesha (NIH/OD) [C] <aesha.brandy@nih.gov>  
**Cc:** Bulls, Michelle G. (NIH/OD) [E] <michelle.bulls@nih.gov>; Meseroll, Rebecca (NIH/OD) [C] <rebecca.meseroll@nih.gov>; Bundesen, Liza (NIH/OD) [E] <lbundese@od.nih.gov>; Kitt, Cheryl (NIH/OD) [E] <kittc@od.nih.gov>; Showe, Melanie (NIH/OD) [E] <showem@od.nih.gov>; Meseroll, Rebecca (NIH/OD) [C] <rebecca.meseroll@nih.gov>; Helfer, Jacqueline (NIH/OD) [C] <jacqueline.helfer@nih.gov>  
**Subject:** FW: Response Creation - DUE by COB Friday April 30th (WF 357204)

Dear Aesha:

Attached is OER's response to the KEI April request to march-in on Xtandi. Mark has also reviewed.

Please let me know if you have any questions.

Ann

**From:** Brandy, Aesha (NIH/OD) [C]  
**Sent:** Tuesday, April 25, 2017 12:23 PM  
**To:** Bulls, Michelle G. (NIH/OD) [E] <michelle.bulls@nih.gov>; Hammersla, Ann (NIH/OD) [E] <hammerslaa@mail.nih.gov>  
**Cc:** Bundesen, Liza (NIH/OD) [E] <lbundese@od.nih.gov>; Kitt, Cheryl (NIH/OD) [E] <kittc@od.nih.gov>; Joshi, Pritty (NIH/OD) [E] <pritty.joshi@nih.gov>; Showe, Melanie (NIH/OD) [E] <showem@od.nih.gov>  
**Subject:** Response Creation - DUE by COB Friday April 30th (WF 357204)

Good Morning Michelle and Ann –

Attached you will find an email, addressed to HHS Secretary Price and DOD Secretary Mattis, from Mr. Andrew Goldman of Knowledge Ecology International. He is writing on behalf of his organization and the Union for Affordable Cancer Treatment, requesting that the government reconsider its decision to not use the march-in rights under the Bayh-Dole Act for the prostate cancer drug Xtandi.

The NIH Exec Sec has assigned this task to both OER and OSP, requesting we work together to draft a Direct Reply response for either Dr. Lauer, Dr. Wolinetz, or whoever else you feel should sign off. It is my understanding that Mark Rohrbaugh, in OSP, has been tracking this and that you may have already had some contact with him regarding this topic.

Would you please work with OSP on this draft and forward me a copy for clearance and submission to the NIH Exec Sec by COB Friday, April 30<sup>th</sup>? I have also attached the supporting documentation for this request.

Please let me know if you have any questions or concerns.

REL0000023994



Thank you for your help with this.

Aesha

\*\*\*\*\*  
\*\*\*\*\*

**Work Folder Information**

**Work Folder:** WF 357204

**Process:** Response Creation

**Program Analyst:** Hurlebaus, Lisa (NIH/OD) [E]

**Due Date:** May 01, 2017

**WF Subject:** OS assignment. KEI & UACT write about the prostate cancer drug, Xtandi (enzalutamide). Asks the Government to reconsider the decision not to use the 'march-in' rights, under the Bayh-Dole Act, for this excessively-priced drug. (AS-760889)

**IC:** od\_oer

**From:** Goldman, Andrew

**To:** Price, TomMattis, Jim

**Remarks:** OS assignment. Note to OER & OSP: Please work together to prepare Direct Reply response. You should decide/recommend who should sign draft response (Dr. Lauer or Dr. Wolinetz; or someone else?).

**b5**

**From:** Paine, Taunton (NIH/OD) [E] [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=E8D5E69B1D824F28B05D62611EC4650B-PAINETT]  
**Sent:** 2/9/2018 3:59:28 PM  
**To:** Bayha, Ryan (NIH/OD) [E] [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=5d5a4353cd514322a8598dbb1751ee79-bayhar]; Jorgenson, Lyric (NIH/OD) [E] [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=3bbde7d361374981a4d336b6eeb17521-jorgensonla]; Rohrbaugh, Mark (NIH/OD) [E] [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=591ab6b2424b4b8997082718cbb29fab-rohrbaum]  
**CC:** Milner, Lauren (NIH/OD) [E] [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=26dd59a437be4f84b46543e144e44773-milnerlc]  
**Subject:** Health Affairs Blog: Drugs Don't Work If People Can't Afford Them: The High Price Of Tisagenlecleucel

See the following story estimating how much CAR-T is overpriced, based in part on estimates of how much NIH funding contributed to its development: <https://www.healthaffairs.org/doi/10.1377/hblog20180205.292531/full/>

## HEALTH AFFAIRS BLOG

# Drugs Don't Work If People Can't Afford Them: The High Price Of Tisagenlecleucel

• Paul Kleutghen David Mitchell Aaron S. Kesselheim Mehdi Najafzadeh Ameet Sarpatwari

FEBRUARY 8, 201810.1377/HBLOG20180205.292531

In a system in which life-saving drugs are developed with direct and indirect taxpayer support and afforded market protection through government-granted exclusivities, patients deserve to know how drug manufacturers are arriving at ever-higher prices for their products. Without such information—and subsequent policy reforms based on it—treatment will become increasingly unaffordable. Sadly, for many Americans, it already has; 21 percent of 1,204 respondents in a December 2016 Henry J. Kaiser Family Foundation survey reported that they or a family member did not fill a prescription in the past year because of cost.

## Why Tisagenlecleucel Matters

One of the most recent examples highlighting the prescription drug price debate is the chimeric antigen receptor T-cell (CAR-T) immunotherapy tisagenlecleucel (Kymriah). Widely hailed as a groundbreaking treatment, it was approved by the Food and Drug Administration (FDA) in August 2017 for the treatment of pediatric and young adult acute lymphoblastic leukemia. In coming years, tisagenlecleucel is expected to receive marketing approval in other countries and for other indications, including adult acute lymphoblastic leukemia, chronic lymphocytic leukemia, diffuse large B-cell lymphoma, and small lymphocytic lymphoma.

Novartis “shattered oncology norms” by setting the price for the one-time infusion of tisagenlecleucel at \$475,000. The company has stated that it will charge only for patients who respond after the first

month of treatment, which would be a little more than 80 percent of those receiving the treatment if the results of clinical trials prove generalizable. Importantly, this price “is just the ‘tip of the iceberg,’” and most patients receiving tisagenlecleucel will require supplementary care such as hospitalization and possible concomitant intravenous infusion of the anti-IL-6-receptor tocilizumab, which is estimated to cost an additional \$500,000.

Is the pricing of tisagenlecleucel justified? The question is personal. Two of us are patients with currently incurable blood cancers, and a CAR-T-based treatment such as tisagenlecleucel could one day represent a life-saving treatment for our diseases. However, if patients like us cannot afford the drug, then this hope is pointless. We therefore sought to analyze whether Novartis’s price was “fair”—as some industry analysts suggest—by comparing the estimated government support in developing tisagenlecleucel and Novartis’s claimed investment with an estimate of the profit Novartis could reap from the drug over the next decade.

## Taxpayer Investment In CAR-T Therapies

Many highly transformative drugs or drug classes approved by the FDA over the past three decades originated in academic or government laboratories with support from public funding primarily through the National Institutes of Health (NIH). The genesis of tisagenlecleucel was no different. Knowledge Ecology International, a not-for-profit, estimates that more than \$200 million in NIH-funded research helped define the innovative CAR-T approach to cancer care. Included in this total was partial support of the pivotal study of tisagenlecleucel in pediatric patients with acute lymphoblastic leukemia that led Novartis’s to take ownership of the drug in 2012. The public investment also occurred when there was the highest risk, while Novartis took its step only after the path to market—and revenues—was clear.

## Novartis’s Profits From Tisagenlecleucel

In attempting to understand the price for tisagenlecleucel, we gathered available data points and otherwise used parameters based on industry practices or Novartis’s claims. Concerning costs, for example, Novartis claims to have spent \$1 billion to bring the drug to market. We could not verify that number but accepted it for this exercise, applying a tax credit for 50 percent of clinical trial costs (the level that had been previously applied to drugs for rare diseases). Carl June, MD, the principal inventor of tisagenlecleucel, placed its production cost at \$20,000 per infusion and noted that this price should decrease as Novartis scaled up production. We nevertheless used a generous \$40,000 production cost per infusion. We applied standard industry royalties that would be due to the University of Pennsylvania, from which Novartis purchased the intellectual property rights to tisagenlecleucel in perpetuity, and relied upon publicly available analyses of royalties paid to Oxford Biomedica, the maker of the viral vector used in tisagenlecleucel. We also accounted for financial support for travel expenses given to 50 percent of patients and added the cost of a registry and surveillance program borne by the company.

Regarding revenue, we estimated that slightly more than 190,000 patients around the world—including 88,458 US patients—would be treated with tisagenlecleucel between 2017 and 2027 based on ongoing clinical trials and disease-specific population data from the National Cancer Institute’s Surveillance, Epidemiology, and End Results program. We believe that our patient estimates are conservative as they reflect that only 30 percent of relapsed/refractory patients will receive tisagenlecleucel treatment, in part owing to projected competition from other market entrants. Although brand-name manufacturers often raise their drug prices each year unless they are facing direct competition, we held our modeled price steady, after discounting for the estimated 16 percent



of patients who clinical trials indicate would not respond in the first 28 days post-infusion and who would not be charged the price of the product, and because Novartis has said it might charge less for other indications. We additionally assumed that Novartis would keep the price of tisagenlecleucel uniform across countries to avoid medical tourism, which would be more feasible for tisagenlecleucel than other treatments given its one-time administration. Our detailed modeling and assumptions can be viewed [here](#).

Based on these inputs, we calculated that Novartis would achieve an average annual operating profit of 84 percent on Kymriah over the next 10 years. After allocating 19 percent of revenue annually for Novartis's historic reported level of research and development—enough to underwrite both its successes and failures—Novartis would still reap a net profit of 65 percent, almost 2.5 times what the company generates on its current product portfolio.

For these reasons, we believe the drug is overpriced. Novartis took a drug developed with significant investment from US taxpayers and is on course to make substantially more than its already healthy performance. We believe Novartis could cover both its historic margins and continuing research and development spending at a retail price of \$160,000.

## What We Want

We believe Americans deserve transparency in the pricing decisions for drugs invented using taxpayer funding. In a health system that accounts for 18 percent of gross domestic product—and in which prescription drugs make up 17 percent of that total—we cannot make wise societal judgements about how to allocate resources without clarity on these matters.

Prescription drugs should be priced in a way that maximizes patient access while ensuring adequate compensation for research and development pipelines and healthy incentives for meaningful innovation. We acknowledge the challenges with such price-setting, but there are similar challenges with the current system that provides monopoly power to drug manufacturers, particularly in an inefficient marketplace in which some payers are restricted in their ability to negotiate.

One potential solution is more visible public input, especially for drugs developed at substantial taxpayer expense. The NIH has the statutory authority to ensure “reasonable terms” for products emerging from intellectual property it has funded. We suggest that the NIH issue a request for information on approaches it and its grantees could use to address downstream pricing issues when working with manufacturers to develop and commercialize a drug. For example, the NIH could create an advisory committee to assist it in negotiating on pricing. Alternatively, the NIH could simply require that patients in the US be charged no more than the average price in six other wealthy nations.

Ultimately, the question cannot only be: How will we pay for this drug? We must also ask: How much profit is fair for a drug that could keep people alive, especially one that US taxpayers supported the foundational science to invent?

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**From:** Berkley, Dale (NIH/OD) [E] [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=5EE461C29F5045A49F0ADF82CAAA2F31-BERKLEYD]  
**Sent:** 6/29/2018 3:49:28 PM  
**To:** Rohrbaugh, Mark (NIH/OD) [E] [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=591ab6b2424b4b8997082718cbb29fab-rohrbaum]  
**Subject:** RE: 83 FR 30448, Prospective Grant of an Exclusive Patent License: Development of an Anti-Mesothelin Chimeric Antigen Receptor (CAR) for the Treatment of Human Cancer

Ok thanks.

**From:** Rohrbaugh, Mark (NIH/OD) [E]  
**Sent:** Friday, June 29, 2018 11:06 AM  
**To:** Berkley, Dale (NIH/OD) [E] <BerkleyD@OD.NIH.GOV>  
**Subject:** RE: 83 FR 30448, Prospective Grant of an Exclusive Patent License: Development of an Anti-Mesothelin Chimeric Antigen Receptor (CAR) for the Treatment of Human Cancer

They have less than 15 days to respond, with the holiday I hope we can have a meeting before then.

b5

b5

**From:** Berkley, Dale (NIH/OD) [E]  
**Sent:** Friday, June 29, 2018 11:02 AM  
**To:** Rohrbaugh, Mark (NIH/OD) [E] <RohrBauM@OD.NIH.GOV>  
**Subject:** RE: 83 FR 30448, Prospective Grant of an Exclusive Patent License: Development of an Anti-Mesothelin Chimeric Antigen Receptor (CAR) for the Treatment of Human Cancer

b5

**From:** Rohrbaugh, Mark (NIH/OD) [E]  
**Sent:** Friday, June 29, 2018 10:59 AM  
**To:** Berkley, Dale (NIH/OD) [E] <BerkleyD@OD.NIH.GOV>  
**Subject:** RE: 83 FR 30448, Prospective Grant of an Exclusive Patent License: Development of an Anti-Mesothelin Chimeric Antigen Receptor (CAR) for the Treatment of Human Cancer

b5

**From:** Berkley, Dale (NIH/OD) [E]  
**Sent:** Friday, June 29, 2018 10:26 AM  
**To:** Rohrbaugh, Mark (NIH/OD) [E] <RohrBauM@OD.NIH.GOV>  
**Subject:** RE: 83 FR 30448, Prospective Grant of an Exclusive Patent License: Development of an Anti-Mesothelin Chimeric Antigen Receptor (CAR) for the Treatment of Human Cancer

b5

**From:** Rohrbaugh, Mark (NIH/OD) [E]  
**Sent:** Friday, June 29, 2018 10:17 AM  
**To:** Berkley, Dale (NIH/OD) [E] <[BerkleyD@OD.NIH.GOV](mailto:BerkleyD@OD.NIH.GOV)>  
**Subject:** FW: 83 FR 30448, Prospective Grant of an Exclusive Patent License: Development of an Anti-Mesothelin Chimeric Antigen Receptor (CAR) for the Treatment of Human Cancer

b5

**From:** Freel, Rose (NIH/NCI) [E]  
**Sent:** Friday, June 29, 2018 9:26 AM  
**To:** Rohrbaugh, Mark (NIH/OD) [E] <[RohrBauM@OD.NIH.GOV](mailto:RohrBauM@OD.NIH.GOV)>  
**Cc:** Rodriguez, Richard (NIH/NCI) [E] <[richard.rodriguez@nih.gov](mailto:richard.rodriguez@nih.gov)>  
**Subject:** FW: 83 FR 30448, Prospective Grant of an Exclusive Patent License: Development of an Anti-Mesothelin Chimeric Antigen Receptor (CAR) for the Treatment of Human Cancer

Hi Mark,

See email below from KEI regarding a FR Notice for intent to grant. Let me know when you have time to discuss.

Thanks!

Rose

--

Rose Santangelo Freel, Ph.D.  
Technology Transfer Manager  
**National Cancer Institute**  
P 301-624-1257 | [rose.freel@nih.gov](mailto:rose.freel@nih.gov)

**From:** James Love [<mailto:james.love@keionline.org>]  
**Sent:** Thursday, June 28, 2018 4:04 PM  
**To:** Freel, Rose (NIH/NCI) [E] <[rose.freel@nih.gov](mailto:rose.freel@nih.gov)>  
**Cc:** Claire Cassedy <[claire.cassedy@keionline.org](mailto:claire.cassedy@keionline.org)>  
**Subject:** 83 FR 30448, Prospective Grant of an Exclusive Patent License: Development of an Anti-Mesothelin Chimeric Antigen Receptor (CAR) for the Treatment of Human Cancer

Rose M. Freel, Ph.D.,  
Licensing and Patenting Manager,  
NCI Technology Transfer Center,  
8490 Progress Drive, Suite 400, Frederick, MD 21701;  
Email: [rose.freel@nih.gov](mailto:rose.freel@nih.gov).

Dear Dr  
Freel,

Has the technology referred to in 83 FR 30448, regarding Development of an Anti-Mesothelin Chimeric Antigen Receptor (CAR) for the Treatment of Human Cancer, been subject to any clinical trials (1) funded by the NIH, or (2) funded by any other party?

This information is useful for KEI in preparing our comments on the license.

Jamie

REL0000023997

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James Love. Knowledge Ecology International

<http://www.keionline.org/donate.html>

KEI DC tel: +1.202.332.2670, US Mobile: +1.202.361.3040, Geneva Mobile: +41.76.413.6584, [twitter.com/jamie\\_love](https://twitter.com/jamie_love)



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**From:** Dick, Taryn (NIH/NCI) [F] [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=D6CF8A6DD6C04487AA59BC4DCA32837E-DICKTE]  
**Sent:** 11/7/2017 1:20:58 PM  
**To:** Rohrbaugh, Mark (NIH/OD) [E] [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=591ab6b2424b4b8997082718cbb29fab-rohrbaum]  
**Subject:** RE: TTC Seminar: Mark Rohrbaugh  
**Attachments:** Joe Allen\_Final.pptx

Hi Mark,

Based on the questions and comments from Joe's talk, I think the NIH TT community would really enjoy another talk on this topic but from an internal perspective.

Just for your reference, I've attached Joe's PPT in case you wanted a reminder as to which topics were already covered and to what extent.

Thanks,  
Taryn

**From:** Rohrbaugh, Mark (NIH/OD) [E]  
**Sent:** Monday, November 6, 2017 3:23 PM  
**To:** Dick, Taryn (NIH/NCI) [F] <taryn.dick@nih.gov>  
**Subject:** RE: TTC Seminar: Mark Rohrbaugh

It will be different from Joe Allen's in that I will focus more internally. Is that ok? Or is this too much like Joe's at NCI. I did not hear the entire talk.

---

**From:** Dick, Taryn (NIH/NCI) [F]  
**Sent:** Monday, November 06, 2017 3:20 PM  
**To:** Rohrbaugh, Mark (NIH/OD) [E] <rohrbaum@od.nih.gov>  
**Subject:** RE: TTC Seminar: Mark Rohrbaugh

Great!

Thank you!

**From:** Rohrbaugh, Mark (NIH/OD) [E]  
**Sent:** Monday, November 6, 2017 3:18 PM  
**To:** Dick, Taryn (NIH/NCI) [F] <taryn.dick@nih.gov>  
**Subject:** RE: TTC Seminar: Mark Rohrbaugh

Exclusive Licensing and Reasonable Pricing Clauses: Why all the fuss?

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**From:** Dick, Taryn (NIH/NCI) [F]  
**Sent:** Monday, November 06, 2017 2:47 PM  
**To:** Rohrbaugh, Mark (NIH/OD) [E] <rohrbaum@od.nih.gov>  
**Subject:** RE: TTC Seminar: Mark Rohrbaugh

Hi Mark,

Hope you had a great weekend. I was just circling back to see if you've settled on a topic? We have our November seminar this Thursday and would like to preview your December talk if possible. Even if you have a tentative title we can use, that will be fine.

Thanks,  
Taryn

**From:** Dick, Taryn (NIH/NCI) [F]  
**Sent:** Monday, October 23, 2017 9:12 AM  
**To:** Rohrbaugh, Mark (NIH/OD) [E] <[RohrBauM@OD.NIH.GOV](mailto:RohrBauM@OD.NIH.GOV)>  
**Subject:** FW: TTC Seminar: Mark Rohrbaugh

Hi Mark,

Hope you had a good weekend.

I just wanted to forward you this email for your consideration for topic ideas for our December Seminar.

Taryn

**From:** Stackhouse, Thomas (NIH/NCI) [E]  
**Sent:** Friday, October 20, 2017 4:22 PM  
**To:** Dick, Taryn (NIH/NCI) [F] <[taryn.dick@nih.gov](mailto:taryn.dick@nih.gov)>  
**Cc:** Salgaller, Michael (NIH/NCI) [E] <[michael.salgaller@nih.gov](mailto:michael.salgaller@nih.gov)>; Newton, Michele (NIH/NCI) [C] <[michele.newton@nih.gov](mailto:michele.newton@nih.gov)>; Maurey, Karen (NIH/NCI) [E] <[maureyk@otd.nci.nih.gov](mailto:maureyk@otd.nci.nih.gov)>  
**Subject:** TTC Seminar: Mark Rohrbaugh

Hi Taryn,

You mentioned that Mark Rohrbaugh, Special Advisor for Technology Transfer, NIH was interested in presenting at a future TTC Seminar and asked if there were any particular topics. One topic raised by some of the TTC staff is the implications of pricing clauses in NIH TT agreements. We got a taste of the topic from our last speaker, Joe Allen. However, it would be interesting to hear NIH's position, some of the ways they are dealing with it, and ways we as a community might be better equipped to understand and response to it if asked.

In addition, I think it would be interesting to hear from Mark on the top 2-3 topics he believes may affect us in the near future as a TT community.

Hope this helpful. Let me know if you have any questions.

Have a great weekend,

Tom



# Bayh-Dole and the Critics: why a Law that Helped save America is under attack

Presented by  
**Joseph Allen**, *President, Allen and Associates*

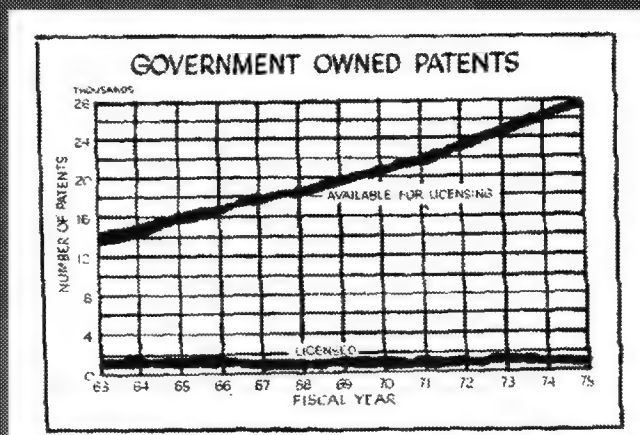


Sen. Birch Bayh (right) and me at Bayh-Dole hearings



## Pre-Bayh-Dole:

Government takes inventions, offers non-exclusive licenses. No drugs developed, less than 4% of inventions licensed





# Bayh-Dole

- Universities, small businesses own federally funded inventions
- Must give preference in licensing to small companies, domestic manufacturers
- Royalties used to reward inventors, fund more R&D, pay tech transfer costs
- Government gets royalty free license
- Agencies can license their inventions

# March in Rights

- Concern dominant companies might license technologies to prevent competition
- Government can march in, requiring universities to issue additional licenses if:
  - Developer not taking effective steps within a reasonable time "to achieve practical application"
  - Licensee cannot meet health or safety needs or requirements for public use
  - Licensee violated domestic manufacturing pledge



## Twenty Two Years Later, Critics invent new meaning for march in's:

- "Paying Twice for the Same Drugs"
  - op-ed in Washington Post, March 27, 2002:
    - alleges Bayh-Dole allows government to march in if price of drug isn't "reasonable"
- Sen. Bayh and Dole reply on April 11, 2002:
  - "Bayh-Dole did not intend that government set prices on resulting products. The law makes no reference to a reasonable price that should be dictated by the government."



# Reasonable pricing march in petitions

- Norvir (2004)
  - Jamie Love petitions NIH because drug "not reasonably priced," misrepresents Bayh-Dole
- Sen. Bayh testifies to NIH:
  - law gives agency no authority to march in on successfully commercialized products, would undermine Bayh-Dole, destroying public/private sector R&D partnerships. If Congress wants price control, must amend law, defining "reasonable price." NIH agrees, dismisses petition
- Latanoprost (glaucoma) 2004:
  - Love says priced higher in US than abroad
  - NIH dismisses as drug commercially available

# Reasonable pricing march-in's

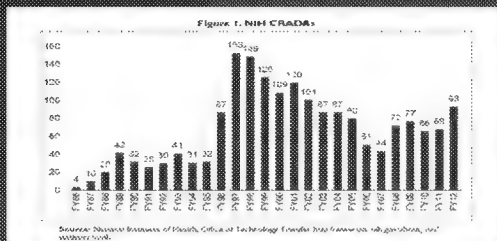
- Norvir (2012)
  - Love petitions NIH again, drug more expensive in US than abroad.
  - NIH dismisses
- Xtandi (2016)
  - Love petitions NIH/DOD, more expensive abroad than US.
  - NIH dismisses, says petitioner's own data shows available for patients.
- HHS Sec. Burwell rebuffs Congressional pressure seeking guidelines for reasonable pricing march in's
  - DOD dismisses KEI's Xtandi petition
- Xtandi, (2017)
  - Love refiles petition with NIH. Congressional letter to Pres. Trump for march in guidelines



# Recent Attacks on Bayh-Dole

- Senators King and Sanders would force "reasonable pricing" provisions onto the system
- Price controls for commercialized products is not authorized by Bayh-Dole
- Sen King would require DOD to misapply the law and reverse its ruling on Xtandi march in petition
- His pricing formula is arbitrary and retroactive, forcing compulsory licensing on those who commercialized federally funded inventions in good faith
- Including pricing restrictions on licenses & CRADAS would have the same impact it had at NIH-- industry partnerships would collapse
- The critics want to return to the pre Bayh-Dole era-- which bitter experience proved fails

# Reasonable pricing at NIH: Agreements plunge, no impact on drug costs

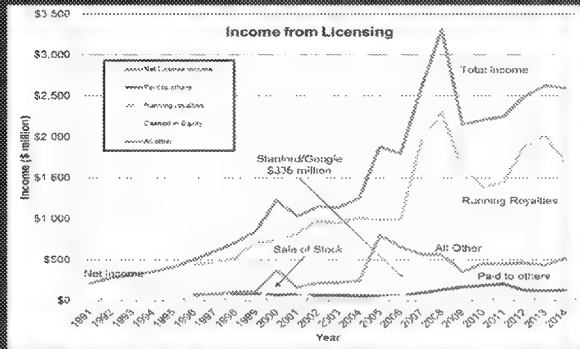


"...the pricing clause has driven industry away from potentially beneficial scientific collaborations with (NIH) scientists without providing an offsetting benefit to the public"

- NIH Director Harold Varmus in '95 revoking the provision— CRADA's up 500% by '97



# Impact of Bayh-Dole



- "Possibly the most inspired piece of legislation to be enacted in America over the past half century"- Economist Tech. Quarterly
- 200 new drugs/vaccines commercialized vs. none before
- 2 new companies/products every day of the year
- \$1.33 Trillion to US economy, 4.2 Million jobs ('96- '15)
- 11,000 startups
- US lead in biotech

# Why do we have this battle?

- Bayh-Dole is opposed by those who believe the patent system is unfair and government funded inventions should be freely available to all
- Some want to “decouple” R&D from markets so governments decide which drugs to commercialize
- Asserting that the public is being gouged resonates with the press and is a good fund raiser
- The critics aren’t going away and we have no choice but to defend ourselves– there’s a lot at stake

# The real success of Bayh-Dole isn't startups, royalties or licenses

Cancer survivor Betsy du Parry hugs Sen. Bayh



"Without your law, I would not be alive today"

---

**From:** Garcia-Perez, Arlyn (NIH/OD) [E] [/O=NIH/OU=NIHEXCHANGE/CN=OD/CN=GARCIAA]  
**Sent:** 6/21/2016 7:40:39 AM  
**To:** Rohrbaugh, Mark (NIH/OD) [E] [/O=NIH/OU=NIHEXCHANGE/cn=OD/cn=ROHRBAUM]  
**Subject:** Re: NIH rejects petition to override patent on pricey prostate cancer drug

Thank you for keeping us in the loop!!!

b5

Arlyn

Sent from my BlackBerry 10 smartphone.

---

**From:** Rohrbaugh, Mark (NIH/OD) [E]  
**Sent:** Monday, June 20, 2016 4:39 PM  
**To:** Baxevanis, Andy (NIH/NHGRI) [E]; Carter, Laura (NIH/OD) [E]; Chloupek, Larry (NIH/OD) [E]; Colbert, Melissa (NIH/OD) [E]; Dearolf, Charles (NIH/OD) [E]; Fonrose, Nadine (NIH/OD) [E]; Garcia-Perez, Arlyn (NIH/OD) [E]; Gottesman, Michael (NIH/OD) [E]; Kleinman, Joe (NIH/OD) [E]; Liu, Paul (NIH/NHGRI) [E]; McBurney, Margaret (NIH/OD) [E]; Milgram, Sharon (NIH/OD) [E]; Owens, Roland (NIH/OD) [E]; Plotz, Paul (NIH/NIAMS) [E]; Roberts, Jacqueline (NIH/OD) [E]; Rohrbaugh, Mark (NIH/OD) [E]; Tobias, Geoffrey (NIH/NCI) [E]; Walters, Judith (NIH/NINDS) [E]; Wanjek, Christopher (NIH/OD) [E]; Wyatt, Richard G (NIH/OD) [E]  
**Subject:** FW: NIH rejects petition to override patent on pricey prostate cancer drug

**From:** Berkson, Laura (NIH/OD) [E]  
**Sent:** Monday, June 20, 2016 4:12 PM  
**To:** Baker, Rebecca (NIH/OD) [E] <bakerrg@od.nih.gov>; Hallett, Adrienne (NIH/OD) [E] <adrienne.hallett@nih.gov>; Myles, Renate (NIH/OD) [E] <mylestr@od.nih.gov>; Rohrbaugh, Mark (NIH/OD) [E] <RohrBauM@OD.NIH.GOV>; Hammersla, Ann (NIH/OD) [E] <hammerslaa@mail.nih.gov>; Culhane, Ned (NIH/OD) [E] <culhanee@mail.nih.gov>; Allen-Gifford, Patrice (NIH/OD) [E] <patrice.allen-gifford@nih.gov>; Higgins, Lauren (NIH/OD) [E] <HigginsL@OD.NIH.GOV>  
**Subject:** NIH rejects petition to override patent on pricey prostate cancer drug

FYI, there is an article in STAT about the Xtandi decision: <https://www.statnews.com/pharmalot/2016/06/20/nih-rejects-patent-petition/>. It links to a pdf of the letter.

## NIH rejects petition to override patent on pricey prostate cancer drug





PAUL MORIGI/GETTY IMAGES

NIH Director Dr. Francis Collins

By ED SILVERMAN *@Pharmainist*  
JUNE 20, 2016

**A**fter five months of deliberation, the US National Institutes of Health on Monday rejected a request by several consumer groups to override the patent on a prostate cancer drug because the medicine is more expensive in the United States than elsewhere. And one of the consumer groups plans to seek an appeal.

Last January, the groups petitioned the NIH to take this step, which is known as a march-in right, to help US patients because federally funded research was used to create Xtandi. The drug is sold by Astellas Pharma and has an average wholesale price in the United States of more than \$129,000, about two to four times more than what other high-income countries are paying, according to the consumer groups.

Under federal law, a march-in right allows an agency that funds private research to require a drug maker to license its patent to another party in order to “alleviate health and safety needs which are not being reasonably satisfied” or when the benefits of a drug are not available on “reasonable terms.” The drug was developed at the University of California, Los Angeles, with grants from NIH and the US

Department of Defense. The school licensed the drug to Medivation, which struck a marketing deal with Astellas.

However, the NIH denied the petition because there was no information to suggest that Xtandi is or will be in short supply, according to a letter sent on Monday by NIH Director Dr. Francis Collins to Knowledge Ecology International, one of the consumer groups. The agency, which has rarely granted such petitions, noted that the litmus test used in one previous case was whether there were sufficient supplies of the medicine for which a petition was sought.

In a statement, the consumer groups argued the NIH “did not evaluate evidence provided that Astellas charges US residents prices that are far higher than those available to non-US consumers, and that price discrimination against US residents is not consistent with making the product ‘available to the public on reasonable terms,’” as required by federal law.

They also maintained the NIH failed to address evidence that “the unreasonably high price for Xtandi limits patient access, places the drugs on restrictive formularies, causes strain to health care budgets, and requires patients to pay unreasonably high coinsurance and copayments,” all of which justify the use of march-in rights.

They added that the NIH ignored its ability to issue a nonexclusive, royalty-free license to allow Xtandi to be manufactured for use by the federal government. Knowledge Ecology legal and policy counselor Andrew Goldman said there is no precondition about supplies and the NIH is wrong to assert that there is no limit on “excessive pricing” in order to grant a march-in right.

“This is contrary to the legislative intent of the law, and sends a terrible signal about the government’s willingness to confront the high drug prices through available legal mechanisms,” he said.

The consumer group plans to submit an appeal to US Secretary of Health and Human Services Sylvia Burwell and said it will base its appeal on the NIH’s “flawed legal rationale” about the use of march-in rights and “its lack of analysis concerning its refusal to use a royalty-free license.” The group added that it plans to refile this case after a new president takes office next year if the HHS declines its appeal.

As part of its effort, Knowledge Ecology two months ago solicited Biolyse, a small Canadian drug company, to make Xtandi. The drug maker maintained it could supply a version for \$3 per 40-milligram tablet, compared with the \$69.41 that Medicare paid in 2014. Biolyse hoped to be able to supply its version in three years. We left word with a company spokesman and will pass along any reply.

An Astellas spokesman wrote us to say that the company is “pleased that the NIH has concluded that Xtandi is broadly available to patients, and we are committed to continuing our work with our diverse stakeholders to provide patients with affordable access to our medicines.”

The rejection is not a surprise, though.

Two months ago, the Obama administration rejected a request from dozens of congressional Democrats, who call themselves the Affordable Drug Pricing Task Force, to develop guidelines that

would require drug makers to license their patents and put a lid on “price gouging.” They argued the NIH should be more aggressively granting march-in rights in light of the high price of medicine.

At the time, Burwell noted such decisions are made on a case-by-case basis. The NIH previously considered using its march-in authority concerning drug pricing in 2004 and 2013, but determined statutory requirements were not met. Two of those instances involved the Norvir AIDS medicine that was marketed by Abbott Laboratories — now owned by AbbVie — and the Xalatan glaucoma treatment sold by Pfizer.

In response, several lawmakers, including presidential aspirant Bernie Sanders, said they would seek a hearing about NIH use of march-in rights, but that never took place.

---

**From:** Hammersla, Ann (NIH/OD) [E] [/O=NIH/OU=NIHEXCHANGE/CN=RECIPIENTS/CN=HAMMERSLAA]  
**Sent:** 5/4/2017 5:09:48 PM  
**To:** Rohrbaugh, Mark (NIH/OD) [E] [/O=NIH/OU=NIHEXCHANGE/cn=OD/cn=ROHRBAUM]  
**Subject:** RE: Final Final Draft  
**Attachments:** Response 05042017 KEI request REV3 ahmr.docx

How is this for the final? Ann

**From:** Rohrbaugh, Mark (NIH/OD) [E]  
**Sent:** Thursday, May 04, 2017 1:01 PM  
**To:** Hammersla, Ann (NIH/OD) [E] <hammerslaa@mail.nih.gov>  
**Subject:** RE: Final Draft

**b5**

**From:** Hammersla, Ann (NIH/OD) [E]  
**Sent:** Thursday, May 04, 2017 12:46 PM  
**To:** Rohrbaugh, Mark (NIH/OD) [E] <[RohrBauM@OD.NIH.GOV](mailto:RohrBauM@OD.NIH.GOV)>  
**Subject:** Final Draft

Mark: Attached is the final draft of the KEI response. I made a couple of small edits to the last draft. Ok? Ann



**b5**

**b5**

---

**From:** Volkov, Marina (NIH/OD) [E] [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=D1B73452D01F4998B0F065C4C0B62449-MVOLKOV]  
**Sent:** 2/1/2018 10:41:40 PM  
**To:** Rohrbaugh, Mark (NIH/OD) [E] [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=591ab6b2424b4b8997082718cbb29fab-rohrbaum]  
**Subject:** Drug Pricing\_LJ (002).docx  
**Attachments:** Drug Pricing\_LJ (002).docx

Hi Mark,

Lyric had a few comments on this brief. If you could respond and send it back to me as soon as possible, would appreciate it.

Thanks!

Marina

**b5**



**b5**

**b5**

**b5**

---

**From:** Joe Allen [jallen@allen-assoc.com]  
**Sent:** 11/20/2017 7:40:07 PM  
**To:** Rohrbaugh, Mark (NIH/OD) [E] [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=591ab6b2424b4b8997082718cbb29fab-rohrbaum]  
**Subject:** Re: NIH on CRISPR licensing

Thanks, very helpful!

On 11/20/2017 12:02 PM, Rohrbaugh, Mark (NIH/OD) [E] wrote:

> Here is the reply

>

> <https://www.keionline.org/23413/>

>

> -----Original Message-----

> From: Joe Allen [mailto:jallen@allen-assoc.com]

> Sent: Monday, November 20, 2017 9:45 AM

> To: Rohrbaugh, Mark (NIH/OD) [E] <rohrbaum@od.nih.gov>

> Subject: NIH on CRISPR licensing

>

> Didn't NIH have a statement replying to KEI's march in petition on CRISPR saying they see no evidence of a problem? If so, could you send me a copy?

>

> Thanks

>

--

Joseph P. Allen  
President  
Allen and Associates  
60704 Rt. 26, South  
Bethesda, OH 43719  
(W) 740-484-1814  
(c) b6  
[www.allen-assoc.com](http://www.allen-assoc.com)



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**From:** Seigfreid, Kim (NIH/OD) [E] [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=918CCB3C48EA40E79A0AB243E5D35298-SEIGFREIDKS]  
**Sent:** 6/13/2018 7:53:54 PM  
**To:** Rohrbaugh, Mark (NIH/OD) [E] [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=591ab6b2424b4b8997082718cbb29fab-rohrbaum]; Myles, Renate (NIH/OD) [E] [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=7d317f5626934585b3692a1823c1b522-mylesr]  
**CC:** Fine, Amanda (NIH/OD) [E] [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=61290b74aa9a44358954c45439ffdeb6-fineab]; Wojtowicz, Emma (NIH/OD) [E] [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=45c6610aca6e44a08d497630425e5ecd-wojtowiczem]  
**Subject:** RE: Worth responding to? NIH CRADA

Would something like this be a fair response? I pulled an example from 2018 to be more current.

b5

**From:** Rohrbaugh, Mark (NIH/OD) [E]  
**Sent:** Wednesday, June 13, 2018 3:20 PM  
**To:** Myles, Renate (NIH/OD) [E] <mylesr@mail.nih.gov>  
**Cc:** Seigfreid, Kim (NIH/OD) [E] <kimberly.seigfreid@nih.gov>; Fine, Amanda (NIH/OD) [E] <amanda.fine@nih.gov>; Wojtowicz, Emma (NIH/OD) [E] <emma.wojtowicz@nih.gov>  
**Subject:** RE: Worth responding to? NIH CRADA

The NIH Policy Chapter says:

b5

**From:** Myles, Renate (NIH/OD) [E]  
**Sent:** Wednesday, June 13, 2018 2:22 PM  
**To:** Rohrbaugh, Mark (NIH/OD) [E] <RohrBauM@OD.NIH.GOV>  
**Cc:** Seigfreid, Kim (NIH/OD) [E] <kimberly.seigfreid@nih.gov>; Fine, Amanda (NIH/OD) [E] <amanda.fine@nih.gov>; Wojtowicz, Emma (NIH/OD) [E] <emma.wojtowicz@nih.gov>  
**Subject:** RE: Worth responding to? NIH CRADA

b5

**From:** Rohrbaugh, Mark (NIH/OD) [E]  
**Sent:** Wednesday, June 13, 2018 2:14 PM  
**To:** Myles, Renate (NIH/OD) [E] <mylesr@mail.nih.gov>  
**Cc:** Seigfreid, Kim (NIH/OD) [E] <kimberly.seigfreid@nih.gov>; Fine, Amanda (NIH/OD) [E] <amanda.fine@nih.gov>

Wojtowicz, Emma (NIH/OD) [E] <[emma.wojtowicz@nih.gov](mailto:emma.wojtowicz@nih.gov)>

**Subject:** RE: Worth responding to? NIH CRADA

I don't know [REDACTED] **b5**

**From:** Rohrbaugh, Mark (NIH/OD) [E]

**Sent:** Tuesday, June 12, 2018 5:30 PM

**To:** Myles, Renate (NIH/OD) [E] <[mylesr@mail.nih.gov](mailto:mylesr@mail.nih.gov)>

**Cc:** Seigfreid, Kim (NIH/OD) [E] <[kimberly.seigfreid@nih.gov](mailto:kimberly.seigfreid@nih.gov)>; Fine, Amanda (NIH/OD) [E] <[amanda.fine@nih.gov](mailto:amanda.fine@nih.gov)>;

Wojtowicz, Emma (NIH/OD) [E] <[emma.wojtowicz@nih.gov](mailto:emma.wojtowicz@nih.gov)>

**Subject:** Re: Worth responding to? NIH CRADA

**b5**

Sent from my iPhone

On Jun 12, 2018, at 4:51 PM, Myles, Renate (NIH/OD) [E] <[mylesr@mail.nih.gov](mailto:mylesr@mail.nih.gov)> wrote:

Looping in Mark Rohrbaugh. Mark: [REDACTED] **b5**

**From:** Seigfreid, Kim (NIH/OD) [E]

**Sent:** Tuesday, June 12, 2018 4:20 PM

**To:** Myles, Renate (NIH/OD) [E] <[mylesr@mail.nih.gov](mailto:mylesr@mail.nih.gov)>

**Subject:** Worth responding to? NIH CRADA

Hi Renate,

[REDACTED] **b5**

[https://twitter.com/jamie\\_love/status/1006496361141940224](https://twitter.com/jamie_love/status/1006496361141940224)


**James Love** Verified account @**jamie\_love**: Not sure if everyone knows, but the @NIHDirector has decided that the @NIH (unlike the Army, Coast Guard, Homeland Security, or the Dept of Commerce) no longer provides public notices of CRADAs. It's part of Collin's minimal transparency policy for NIH owned IP.


Kim

REL0000024015

Kim Seigfreid  
Public Affairs Specialist  
Office of Communications and Public Liaison  
National Institutes of Health  
Building 1, Room 336  
ph: 301-435-3639  
fx: 301-496-0017

[kim.seigfreid@nih.gov](mailto:kim.seigfreid@nih.gov)

Follow the NIH Director on  [Twitter](#) and  [Email Updates](#)

Follow the NIH on  [Facebook](#),  [Twitter](#), and  [YouTube](#)

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**From:** Kassilke, Deborah (NIH/OD) [E] [/O=NIH/OU=NIEXCHANGE/CN=OD/CN=KASSILKE]  
**Sent:** 6/20/2016 8:49:32 PM  
**To:** Rohrbaugh, Mark (NIH/OD) [E] [/O=NIH/OU=NIEXCHANGE/cn=OD/cn=ROHRBAUM]  
**CC:** Rogers, Karen (NIH/OD) [E] [/O=NIH/OU=NIEXCHANGE/cn=OD/cn=GarrettK]; Hammersla, Ann (NIH/OD) [E] [/O=NIH/OU=NIEXCHANGE/cn=Recipients/cn=hammerslaa]  
**Subject:** RE: AJOY?: NIH rejects petition to override patent on pricey prostate cancer drug

b5

**From:** Rohrbaugh, Mark (NIH/OD) [E]  
**Sent:** Monday, June 20, 2016 4:43 PM  
**To:** Kassilke, Deborah (NIH/OD) [E] <deborah.kassilke@nih.gov>  
**Cc:** Rogers, Karen (NIH/OD) [E] <RogersK@od.nih.gov>; Hammersla, Ann (NIH/OD) [E] <hammerslaa@mail.nih.gov>  
**Subject:** RE: AJOY?: NIH rejects petition to override patent on pricey prostate cancer drug

b5

**From:** Kassilke, Deborah (NIH/OD) [E]  
**Sent:** Monday, June 20, 2016 4:39 PM  
**To:** Rohrbaugh, Mark (NIH/OD) [E] <RohrBauM@OD.NIH.GOV>  
**Cc:** Rogers, Karen (NIH/OD) [E] <RogersK@od.nih.gov>  
**Subject:** FW: AJOY?: NIH rejects petition to override patent on pricey prostate cancer drug

b5

**From:** Prabhu, Ajoy (NIH/OD) [E]  
**Sent:** Monday, June 20, 2016 4:38 PM  
**To:** Kassilke, Deborah (NIH/OD) [E] <deborah.kassilke@nih.gov>  
**Cc:** Rogers, Karen (NIH/OD) [E] <RogersK@od.nih.gov>  
**Subject:** Re: AJOY?: NIH rejects petition to override patent on pricey prostate cancer drug

b5

Ajoy

---

**From:** "Kassilke, Deborah (NIH/OD) [E]" <deborah.kassilke@nih.gov>  
**Date:** Monday, June 20, 2016 at 4:34 PM  
**To:** Ajoy Prabhu <aprabhu@od.nih.gov>  
**Cc:** Karen Rogers <RogersK@od.nih.gov>  
**Subject:** RE: AJOY?: NIH rejects petition to override patent on pricey prostate cancer drug

REL0000024016



Ajoy —

b5

b5

**From:** Prabhu, Ajoy (NIH/OD) [E]  
**Sent:** Monday, June 20, 2016 4:29 PM  
**To:** Kassilke, Deborah (NIH/OD) [E] <[deborah.kassilke@nih.gov](mailto:deborah.kassilke@nih.gov)>  
**Cc:** Rogers, Karen (NIH/OD) [E] <[RogersK@od.nih.gov](mailto:RogersK@od.nih.gov)>  
**Subject:** Re: AJOY?: NIH rejects petition to override patent on pricey prostate cancer drug

Sorry about the delay. It is up on the page—

<http://www.ott.nih.gov/policies-reports>

Exact URL is: [http://www.ott.nih.gov/sites/default/files/documents/policy/pdfs/Final\\_Response\\_Goldman\\_6.20.2016.pdf](http://www.ott.nih.gov/sites/default/files/documents/policy/pdfs/Final_Response_Goldman_6.20.2016.pdf)

Ajoy

---

**From:** "Kassilke, Deborah (NIH/OD) [E]" <[deborah.kassilke@nih.gov](mailto:deborah.kassilke@nih.gov)>  
**Date:** Monday, June 20, 2016 at 4:23 PM  
**To:** Ajoy Prabhu <[aprabhu@od.nih.gov](mailto:aprabhu@od.nih.gov)>  
**Cc:** Karen Rogers <[RogersK@od.nih.gov](mailto:RogersK@od.nih.gov)>  
**Subject:** RE: AJOY?: NIH rejects petition to override patent on pricey prostate cancer drug

b5

**From:** Prabhu, Ajoy (NIH/OD) [E]  
**Sent:** Monday, June 20, 2016 4:23 PM  
**To:** Kassilke, Deborah (NIH/OD) [E] <[deborah.kassilke@nih.gov](mailto:deborah.kassilke@nih.gov)>  
**Cc:** Rogers, Karen (NIH/OD) [E] <[RogersK@od.nih.gov](mailto:RogersK@od.nih.gov)>  
**Subject:** Re: AJOY?: NIH rejects petition to override patent on pricey prostate cancer drug

Not at all. I will certainly make sure that it is up shortly. I had to check with Promet about the security update that was scheduled over the weekend.

Ajoy

---

**From:** "Kassilke, Deborah (NIH/OD) [E]" <[deborah.kassilke@nih.gov](mailto:deborah.kassilke@nih.gov)>  
**Date:** Monday, June 20, 2016 at 4:20 PM  
**To:** Ajoy Prabhu <[aprabhu@od.nih.gov](mailto:aprabhu@od.nih.gov)>  
**Cc:** Karen Rogers <[RogersK@od.nih.gov](mailto:RogersK@od.nih.gov)>  
**Subject:** AJOY?: NIH rejects petition to override patent on pricey prostate cancer drug

**From:** Rohrbaugh, Mark (NIH/OD) [E]  
**Sent:** Monday, June 20, 2016 4:20 PM  
**To:** Rogers, Karen (NIH/OD) [E] <[RogersK@od.nih.gov](mailto:RogersK@od.nih.gov)>; Kassilke, Deborah (NIH/OD) [E] <[deborah.kassilke@nih.gov](mailto:deborah.kassilke@nih.gov)>  
**Subject:** FW: NIH rejects petition to override patent on pricey prostate cancer drug

Sorry to bug you.. would be great to get the decision up today.

REL0000024016

**From:** Berkson, Laura (NIH/OD) [E]

**Sent:** Monday, June 20, 2016 4:12 PM

**To:** Baker, Rebecca (NIH/OD) [E] <bakerrg@od.nih.gov>; Hallett, Adrienne (NIH/OD) [E] <adrienne.hallett@nih.gov>; Myles, Renate (NIH/OD) [E] <mylesr@od.nih.gov>; Rohrbaugh, Mark (NIH/OD) [E] <RohrBauM@OD.NIH.GOV>; Hammersla, Ann (NIH/OD) [E] <hammerslaa@mail.nih.gov>; Culhane, Ned (NIH/OD) [E] <culhanee@mail.nih.gov>; Allen-Gifford, Patrice (NIH/OD) [E] <patrice.allen-gifford@nih.gov>; Higgins, Lauren (NIH/OD) [E] <HigginsL@OD.NIH.GOV>

**Subject:** NIH rejects petition to override patent on pricey prostate cancer drug

FYI, there is an article in STAT about the Xtandi decision: <https://www.statnews.com/pharmalot/2016/06/20/nih-rejects-patent-petition/>. It links to a pdf of the letter.

## NIH rejects petition to override patent on pricey prostate cancer drug



PAUL MORIGI/GETTY IMAGES

NIH Director Dr. Francis Collins

By ED SILVERMAN *@EdSilverman*

JUNE 20, 2016



After five months of deliberation, the US National Institutes of Health on Monday rejected a request by several consumer groups to override the patent on a prostate cancer drug because the medicine is more expensive in the United States than elsewhere. And one of the consumer groups plans to seek an appeal.

Last January, the groups petitioned the NIH to take this step, which is known as a march-in right, to help US patients because federally funded research was used to create Xtandi. The drug is sold by Astellas Pharma and has an average wholesale price in the United States of more than \$129,000, about two to four times more than what other high-income countries are paying, according to the consumer groups.

Under federal law, a march-in right allows an agency that funds private research to require a drug maker to license its patent to another party in order to “alleviate health and safety needs which are not being reasonably satisfied” or when the benefits of a drug are not available on “reasonable terms.” The drug was developed at the University of California, Los Angeles, with grants from NIH and the US Department of Defense. The school licensed the drug to Medivation, which struck a marketing deal with Astellas.

However, the NIH denied the petition because there was no information to suggest that Xtandi is or will be in short supply, according to a letter sent on Monday by NIH Director Dr. Francis Collins to Knowledge Ecology International, one of the consumer groups. The agency, which has rarely granted such petitions, noted that the litmus test used in one previous case was whether there were sufficient supplies of the medicine for which a petition was sought.

In a statement, the consumer groups argued the NIH “did not evaluate evidence provided that Astellas charges US residents prices that are far higher than those available to non-US consumers, and that price discrimination against US residents is not consistent with making the product ‘available to the public on reasonable terms,’” as required by federal law.

They also maintained the NIH failed to address evidence that “the unreasonably high price for Xtandi limits patient access, places the drugs on restrictive formularies, causes strain to health care budgets, and requires patients to pay unreasonably high coinsurance and copayments,” all of which justify the use of march-in rights.

They added that the NIH ignored its ability to issue a nonexclusive, royalty-free license to allow Xtandi to be manufactured for use by the federal government. Knowledge Ecology legal and policy counselor Andrew Goldman said there is no precondition about supplies and the NIH is wrong to assert that there is no limit on “excessive pricing” in order to grant a march-in right.

“This is contrary to the legislative intent of the law, and sends a terrible signal about the government’s willingness to confront the high drug prices through available legal mechanisms,” he said.

The consumer group plans to submit an appeal to US Secretary of Health and Human Services Sylvia Burwell and said it will base its appeal on the NIH’s “flawed legal rationale” about the use of march-in

rights and “its lack of analysis concerning its refusal to use a royalty-free license.” The group added that it plans to refile this case after a new president takes office next year if the HHS declines its appeal.

As part of its effort, Knowledge Ecology two months ago solicited Biolyse, a small Canadian drug company, to make Xtandi. The drug maker maintained it could supply a version for \$3 per 40-milligram tablet, compared with the \$69.41 that Medicare paid in 2014. Biolyse hoped to be able to supply its version in three years. We left word with a company spokesman and will pass along any reply.

An Astellas spokesman wrote us to say that the company is “pleased that the NIH has concluded that Xtandi is broadly available to patients, and we are committed to continuing our work with our diverse stakeholders to provide patients with affordable access to our medicines.”

The rejection is not a surprise, though.

Two months ago, the Obama administration rejected a request from dozens of congressional Democrats, who call themselves the Affordable Drug Pricing Task Force, to develop guidelines that would require drug makers to license their patents and put a lid on “price gouging.” They argued the NIH should be more aggressively granting march-in rights in light of the high price of medicine.

At the time, Burwell noted such decisions are made on a case-by-case basis. The NIH previously considered using its march-in authority concerning drug pricing in 2004 and 2013, but determined statutory requirements were not met. Two of those instances involved the Norvir AIDS medicine that was marketed by Abbott Laboratories — now owned by AbbVie — and the Xalatan glaucoma treatment sold by Pfizer.

In response, several lawmakers, including presidential aspirant Bernie Sanders, said they would seek a hearing about NIH use of march-in rights, but that never took place.





Mark: Attached is the final draft of the KEI response. I made a couple of small edits to the last draft. Ok? Ann

**b5**

**b5**



---

**From:** Rodriguez, Richard (NIH/NCI) [E] [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=8092CB5394E04733AC0D4D84D25F65E5-RODRIGR]  
**Sent:** 8/20/2018 1:43:09 PM  
**To:** Rohrbaugh, Mark (NIH/OD) [E] [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=591ab6b2424b4b8997082718cbb29fab-rohrbaum]  
**Subject:** A-423-2018\_Preliminary Determination\_draft 8-17-2018.docx  
**Attachments:** A-423-2018\_Preliminary Determination\_draft 8-17-2018.docx

Hi Mark,

**b5**

Richard

**b5**

**b5**

**b5**

**b5**



**b5**

**b5**

**b5**

**b5**

**b5**



**b5**

**b5**

**b5**

**b5**

**b5**



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**From:** Dodson, Sara (NIH/OD) [E] [/O=NIH/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=DODSONSE]  
**Sent:** 6/16/2017 8:24:06 PM  
**To:** Rohrbaugh, Mark (NIH/OD) [E] [/O=NIH/OU=NIH/EXCHANGE/cn=OD/cn=ROHRBAUM]  
**Subject:** RE: final memo to FC on royalties

Here's my best hunch for where you wanted that language inserted:

**b5**

---

**From:** Rohrbaugh, Mark (NIH/OD) [E]  
**Sent:** Friday, June 16, 2017 4:12 PM  
**To:** Dodson, Sara (NIH/OD) [E] <sara.dodson@nih.gov>  
**Subject:** Re: final memo to FC on royalties

Can you see his edits from the original?

Sent from my iPhone

On Jun 16, 2017, at 2:59 PM, Dodson, Sara (NIH/OD) [E] <sara.dodson@nih.gov> wrote:

Sure, Mark. I'm taking a look at this now.

---

**From:** Rohrbaugh, Mark (NIH/OD) [E]  
**Sent:** Friday, June 16, 2017 3:43 PM  
**To:** Dodson, Sara (NIH/OD) [E] <sara.dodson@nih.gov>  
**Cc:** Jorgenson, Lyric (NIH/OD) [E] <jorgensonla@od.nih.gov>  
**Subject:** Fwd: final memo to FC on royalties

Sorry to bug you late on Friday but could you try and edit this today based on my comments. I will send you Dale's edits separately

Sent from my iPhone

Begin forwarded message:

**From:** "Rohrbaugh, Mark (NIH/OD) [E]" <RohrBauM@OD.NIH.GOV>  
**Date:** June 16, 2017 at 2:28:40 PM CDT  
**To:** "Berkley, Dale (NIH/OD) [E]" <BerkleyD@OD.NIH.GOV>  
**Cc:** "Jorgenson, Lyric (NIH/OD) [E]" <jorgensonla@od.nih.gov>, "Hammersla, Ann (NIH/OD) [E]" <hammerslaa@mail.nih.gov>  
**Subject:** Re: final memo to FC on royalties

**b5**

The challenge to edit it is that I am traveling all day and can only work on my iPhone.

Sent from my iPhone

On Jun 16, 2017, at 3:09 PM, Berkley, Dale (NIH/OD) [E] <[BerkleyD@OD.NIH.GOV](mailto:BerkleyD@OD.NIH.GOV)> wrote:

**b5**

Dale D. Berkley, Ph.D., J.D.  
Office of the General Counsel, PHD, NIH Branch  
Bldg. 31, Rm. 47  
Bethesda, MD 20892  
301-496-6043  
301-402-2528(Fax)

This message is intended for the exclusive use of the recipient(s) named above. It may contain information that is PROTECTED or PRIVILEGED, and it should not be disseminated, distributed, or copied to persons not authorized to receive such information.

**From:** Rohrbaugh, Mark (NIH/OD) [E]  
**Sent:** Friday, June 16, 2017 3:07 PM  
**To:** Berkley, Dale (NIH/OD) [E] <[BerkleyD@OD.NIH.GOV](mailto:BerkleyD@OD.NIH.GOV)>  
**Cc:** Jorgenson, Lyric (NIH/OD) [E] <[jorgensonla@od.nih.gov](mailto:jorgensonla@od.nih.gov)>;  
Hammersla, Ann (NIH/OD) [E] <[hammerslaa@mail.nih.gov](mailto:hammerslaa@mail.nih.gov)>  
**Subject:** Re: final memo to FC on royalties

**b5**

Sent from my iPhone

On Jun 16, 2017, at 11:26 AM, Berkley, Dale (NIH/OD) [E] <[BerkleyD@OD.NIH.GOV](mailto:BerkleyD@OD.NIH.GOV)> wrote:

Attached are a few edits and comments for your consideration.

Best, Dale

Dale D. Berkley, Ph.D., J.D.  
Office of the General Counsel, PHD, NIH Branch  
Bldg. 31, Rm. 47  
Bethesda, MD 20892  
301 496 6043  
301-402-2528(Fax)

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**From:** Jorgenson, Lyric (NIH/OD) [E]  
**Sent:** Friday, June 16, 2017 10:07 AM  
**To:** Berkley, Dale (NIH/OD) [E]  
<BerkleyD@OD.NIH.GOV>  
**Cc:** Rohrbaugh, Mark (NIH/OD) [E]  
<RohrBauM@OD.NIH.GOV>  
**Subject:** FW: final memo to FC on royalties

Hi Dale,

Let me apologize in advance for being needy— I believe Mark Rohrbaugh sent you a copy of this draft response to KEI regarding CRISPR royalties. We are trying to get it under Francis' review today before Carrie leaves town. Did you have a chance to review?

Again – I apologize for the turn around.

Best,

Lyric

**From:** Rohrbaugh, Mark (NIH/OD) [E]  
**Sent:** Monday, May 15, 2017 3:19 PM  
**To:** Jorgenson, Lyric (NIH/OD) [E]  
<jorgensonla@od.nih.gov>  
**Subject:** final memo to FC on royalties

Mark L. Rohrbaugh, Ph.D., J.D.  
Special Advisor for Technology Transfer  
Director, Division of Technology Transfer and  
Innovation Policy  
Office of Science Policy  
Office of the Director  
National Institutes of Health

<WF365656 KEI Response OEROSP (002)--  
OGCBerkleyComments.docx>

REL0000024021

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**From:** Volkov, Marina (NIH/OD) [E] [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=D1B73452D01F4998B0F065C4C0B62449-MVOLKOV]  
**Sent:** 1/31/2018 9:23:59 PM  
**To:** Rohrbaugh, Mark (NIH/OD) [E] [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=591ab6b2424b4b8997082718cbb29fab-rohrbaum]  
**Subject:** Drug Pricing\_mv edits.docx  
**Attachments:** Drug Pricing\_mv edits.docx

Hi Mark,

b5

 I took it out of track changes. Please take a look and let me know if what I did makes sense, and if there is anything you would like to add.

We are really late with this one (sorry, my fault!), and Lyric still needs to review it, so would appreciate it if I could get it back as soon as possible.

Thanks,

Marina

**b5**



**b5**

**b5**

**b5**

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**From:** Berkson, Laura (NIH/OD) [E] [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=ADB561AB47E54FDC94E2A54682514434-BERKSONLD]  
**Sent:** 11/17/2017 6:44:58 PM  
**To:** Rohrbaugh, Mark (NIH/OD) [E] [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=591ab6b2424b4b8997082718cbb29fab-rohrbaum]  
**Subject:** STAT: A plan to develop a Zika vaccine runs into controversy, as questions swirl about price and conflicts of interest

FYI. <https://www.statnews.com/pharmalot/2017/11/17/nih-zika-vaccine-conflicts/>

## A plan to develop a Zika vaccine runs into controversy, as questions swirl about price and conflicts of interest

By [Ed Silverman @Pharmalot](#)

November 17, 2017

Yet another battle may erupt over a Zika virus vaccine and the extent to which drug makers should be allowed to benefit from products that are developed — at least in part — with taxpayer funds. There is an added twist in this case, however, in the form of potential conflicts of interest.

Let's start at the top: Last month, the National Institutes of Health [announced](#) plans to issue an exclusive license to a privately held company called PaxVax to develop a Zika vaccine. Few details were disclosed, but the move is now prompting demands from advocacy groups that the federal government should not award an exclusive license or, if it does, should ensure that any vaccine is priced so it is accessible to Americans.

The concern is that the federal government will award PaxVax an effective monopoly on the vaccine technology for at least seven years, along with valuable tax credits, while failing to receive any guarantees from the company that a vaccine would be affordable.

In their comments to the NIH, the advocacy groups argued that PaxVax may be eligible for tax credits under the Orphan Drug Act amounting to 50 percent of the costs of clinical trial work. They also say the company could get seven years of exclusive marketing rights under the same law and a priority review voucher, which entitles companies to faster regulatory product reviews and can be sold to other drug companies.

"There is considerable evidence that the grant of exclusivity is not a reasonable and necessary incentive to promote innovation and further development of a Zika vaccine," wrote Doctors Without Borders and Knowledge Ecology International in [comments](#) filed with the NIH. "...An exclusive license can be a barrier to ensuring a Zika vaccine will be available and affordable to all who need it."

[Related Story:](#)

[Is there a future for a Zika vaccine?](#)

The same complaints were voiced this past summer when the U.S. Army sought to strike a deal with Sanofi([SNY](#)) for another Zika vaccine that was being developed, in part, with taxpayer funds. Several lawmakers and advocacy groups pressed the Army to eschew an exclusive license or, if that was not possible, obtain a guarantee over affordable pricing.

Sanofi refused to provide a pricing guarantee, although later denied doing so. In any case, its work was halted two months ago, when the Biomedical Advanced Research and Development Authority, an arm of the U.S. Department of Health and Human Services ended its financial assistance for its vaccine.

PaxVax, however, appears mindful of the negative publicity that plagued Sanofi.

A spokeswoman sent us a statement saying it is “premature” to set a price for its Zika vaccine and that “commercialization decisions will not be made for years – and only if we are successful in developing the product. Should we succeed in completing data and regulatory milestones, PaxVax is committed to working with stakeholders to ensure fair pricing and access to the vaccine.”

However, she declined to comment on talks with the NIH or say how “fair pricing” is being defined. When asked for more detail, she wrote back to say that PaxVax is “committed to finding a way to provide access to those who need these vaccines most.”

In general, manufacturers are reluctant to commit to pricing terms while such projects are in the early stages of development. For this reason, the National Institutes of Health in 1995 removed what were called “reasonable pricing” clauses from cooperative R&D agreements. At the time, former NIH director Dr. Harold Varmus described such clauses as a “restraint” on new product development.

In comments to the NIH about a PaxVax license, the advocacy groups were specific about pricing.

They want PaxVax to agree to market a vaccine in the U.S. at a price no higher than the median price charged in other countries with large a gross domestic product and at least half of U.S. per capita income. They also want PaxVax to disclose steps it will take to make a vaccine available at an affordable price in countries with demonstrated need.

In a note to us, an NIH spokeswoman did not directly address pricing concerns, but did write that “no other company has submitted an application to license this vaccine candidate — a necessary step for furthering its development and realizing the benefits of the significant taxpayer investment that has been made to date.” She added the agency will consider comments before continuing the process.

We should note that, last May, U.S. Army Acting Secretary Robert Speer pointed out that only Sanofi was “willing to license” the particular discovery that formed the basis for the Zika work that would have been licensed to the company. This prompted speculation among lawmakers and patient advocates that the Army was unwilling to push Sanofi about pricing over fears it would walk away.

Whether a similar scenario plays out here is unclear.

There is a potentially complicating factor, however, in this latest case.

The KEI advocacy group, which was active in pushing the Army to extract better licensing terms from Sanofi, filed a separate comment with the NIH to say that an exclusive license with PaxVax has the “appearance of a conflict of interest.”

Two years ago, Cerberus Capital Management spent \$105 million to purchase a majority interest in PaxVax. Stephen Feinberg, who heads the fund, donated about \$1.5 million to Rebuild America Now, a Super PAC that supported President Donald Trump.

Also, Ken Kelley, who founded and previously ran PaxVax, has been working as a White House Presidential Executive Fellow since 2015. According to his LinkedIn page, Kelley is advising the BARDA director as well as the director of the Vaccine Research Center of the National Institute of Allergy and Infectious Diseases, which is the NIH unit considering whether to issue an exclusive license to PaxVax.

"The potential of a conflict of interest between interested parties in PaxVax and parties within the federal government calls for a higher level of transparency into the transfer of any publicly funded technology," KEI said in a statement.

## About the Author



Ed Silverman

Pharmalot Columnist, Senior Writer

Ed covers the pharmaceutical industry.

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**From:** Lambertson, David (NIH/NCI) [E] [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=3C95B34F709746A8A2553CE54E74ACE2-LAMBERTSOND]  
**Sent:** 7/16/2019 5:06:24 PM  
**To:** Rohrbaugh, Mark (NIH/OD) [E] [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=591ab6b2424b4b8997082718cbb29fab-rohrbaum]  
**Subject:** FW: Cost of manufacturing CARs

Hi Mark,

A received a second inquiry from KEI regarding cost of manufacturing., so Richard wanted me to check with you regarding a response. b5

Thanks,  
Dave

David A. Lambertson, Ph.D.  
Senior Technology Transfer Manager  
Technology Transfer Center  
National Cancer Institute/NIH  
[david.lambertson@nih.gov](mailto:david.lambertson@nih.gov)  
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**From:** James Love <james.love@keionline.org>  
**Sent:** Tuesday, July 16, 2019 12:45 PM  
**To:** Lambertson, David (NIH/NCI) [E] <david.lambertson@nih.gov>  
**Cc:** Manon Ress <MANON.RESS@cancerunion.org>; Claire Cassedy <claire.cassedy@keionline.org>; Kathryn Ardizzone <kathryn.ardizzone@keionline.org>; Luis Gil Abinader <luis.gil.abinader@keionline.org>; Collins, Francis (NIH/OD) [E] <collinsf@od.nih.gov>  
**Subject:** Cost of manufacturing CARs

David A. Lambertson, Ph.D.  
  
Senior Technology Transfer Manager  
  
Technology Transfer Center  
National Cancer Institute/NIH  
[david.lambertson@nih.gov](mailto:david.lambertson@nih.gov)

<http://ttc.nci.nih.gov/>

Phone (direct): (240) 276-6467

Dear Dr. Lambertson,

The proposed license for the Kite CAR patents involve technology that should be "available to the public on reasonable terms." In this regard, we are interested in know what it costs the NIH to manufacture CAR T cells.

Can you provide KEI with the actual or estimated costs of manufacturing the cells for the inventions to be licensed, and also, provide us with any additional information the NIH has regarding costs of manufacturing CAR T cells?

James Love

--

James Love. Knowledge Ecology International

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<http://www.keionline.org>

[twitter.com/jamie\\_love](https://twitter.com/jamie_love)

---

**From:** Yang, Jasmine (NIH/NCI) [E] [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=DCACD5B675E74725A0D6A6FC9A130431-YANGJJ2\_6B5]  
**Sent:** 1/3/2019 2:38:40 PM  
**To:** Rohrbaugh, Mark (NIH/OD) [E] [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=591ab6b2424b4b8997082718cbb29fab-rohrbaum]  
**CC:** Rodriguez, Richard (NIH/NCI) [E] [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=8092cb5394e04733ac0d4d84d25f65e5-rodrigr]; Berkley, Dale (NIH/OD) [E] [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=5ee461c29f5045a49f0adf82caaa2f31-berkleyd]  
**Subject:** RE: KEI comment to intent to grant A-066-2019  
**Attachments:** 20170817\_PublishedPCTApplication\_WO2017139758.pdf; Ltr to KEI\_2019-01-03.docx

Thanks for the feedback. I have made changes as asked.

b5

b5

Let me know if additional changes are needed.

Best,  
Jasmine

**From:** Rohrbaugh, Mark (NIH/OD) [E]  
**Sent:** Wednesday, January 2, 2019 3:37 PM  
**To:** Yang, Jasmine (NIH/NCI) [E] <jasmine.yang@nih.gov>  
**Cc:** Rodriguez, Richard (NIH/NCI) [E] <richard.rodriguez@nih.gov>; Berkley, Dale (NIH/OD) [E] <BerkleyD@OD.NIH.GOV>  
**Subject:** RE: KEI comment to intent to grant A-066-2019

Jasmine:

Dale and I have review this and suggest

b5

b5

Mark

**From:** Yang, Jasmine (NIH/NCI) [E] <jasmine.yang@nih.gov>  
**Sent:** Wednesday, January 02, 2019 1:38 PM  
**To:** Rohrbaugh, Mark (NIH/OD) [E] <rohrbaum@od.nih.gov>; Berkley, Dale (NIH/OD) [E] <berkleyd@od.nih.gov>  
**Cc:** Rodriguez, Richard (NIH/NCI) [E] <richard.rodriguez@nih.gov>  
**Subject:** KEI comment to intent to grant A-066-2019

Hello,

I have received the attached emails from Jaime Love of KEI in response to the following FRN  
<https://www.federalregister.gov/documents/2018/12/21/2018-27671/prospective-grant-of-an-exclusive-patent-license-multifunctional-rna-nanoparticles-and-methods-of>.

Attached is a draft response that I intend to respond with, pending your comments/feedback. Please let me know if anything needs to be changed to the draft and if you have any questions regarding the company, Sixfold, or the technology.

Thank you,  
Jasmine

---

Jasmine J. Yang, Ph.D.

REL0000024031

Sr. Technology Transfer Manager  
Technology Transfer Center - National Cancer Institute | The National Institutes of Health  
Riverade 3, Suite 400 | 8400 Progress Drive - Frederick, MD 21701  
Email: [jasmine.yang@nih.gov](mailto:jasmine.yang@nih.gov) | P: 301 634 8773 | F: 301 634 3027  
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(51) International Patent Classification:

C12N 15/113 (2010.01) A61K 47/55 (2017.01)  
B82Y 5/00 (2011.01) C12N 15/87 (2006.01)

(21) International Application Number:

PCT/US2017/017661

(22) International Filing Date:

13 February 2017 (13.02.2017)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

62/294,848 12 February 2016 (12.02.2016) US

(71) Applicant: **THE UNITED STATES OF AMERICA**, as represented by **THE SECRETARY, DEPARTMENT OF HEALTH & HUMAN SERVICES** [US/US]; National Institute of Health, Office of Technology Transfer, 6011 Executive Boulevard, Suite 325, Rockville, MD 20852 (US).

(72) Inventors: **SHAPIRO, Bruce, Allen**; 8220 Langport Terrace, Gaithersburg, MD 20877 (US). **AFONIN, Kirill, Andreevich**; 5839 Brachnell View Drive, Charlotte, NC 28269 (US). **VIARD, Mathias, D.**; Building 469, Room 211, Basic Research Program, Saic-Frederick, CCRNP, Frederick, MD 21702 (US). **BINDEWALD, Eckart, H.U.**; 18 Victoria Sq., Frederick, MD 21702 (US).

(74) Agents: **MCCOOL, Gabriel, J.** et al.; Locke Lord LLP, P.O. Box 55874, Boston, MA 02205 (US).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

- with international search report (Art. 21(3))
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))

(54) Title: RNA/DNA HYBRID NANOPARTICLES MODIFIED WITH SINGLE STRANDED RNA TOEHOLDS AND USES THEREOF

(57) Abstract: The invention discloses the use of single- stranded RNA toeholds of different lengths to promote the re-association of various RNA-DNA hybrids, which results in activation of multiple split functionalities inside human cells. Previously designed RNA/DNA nanoparticles employed single- stranded DNA toeholds to initiate re-association. The use of RNA toeholds is advantageous because of the simpler design rules, the shorter toeholds, and the smaller size of the resulting nanoparticles compared to the same hybrid nanoparticles with single-stranded DNA toeholds. Moreover, the co-transcriptional assemblies result in higher yields for hybrid nanoparticles with ssRNA toeholds.

**TITLE****RNA/DNA HYBRID NANOPARTICLES MODIFIED WITH SINGLE STRANDED  
RNA TOEHOLDS AND USES THEREOF****GOVERNMENT FUNDING**

Research supporting this application was carried out by the United States of America as represented by the Secretary, Department of Health and Human Services. The government has certain rights in the invention.

**CROSS-REFERENCE TO RELATED APPLICATIONS**

This application claims priority to U.S. Provisional Patent Application No. 62/294,848, filed February 12, 2016.

**INCORPORATION BY REFERENCE**

This application generally relates to the design, preparation, and therapeutic/diagnostic application of specialized RNA/DNA nanoparticles which comprise single-stranded RNA (“ssRNA”) toehold sequences that improve their structure and function as described here. In general and where applicable, this specification incorporates by reference in their entireties Applicant’s prior patent applications relating to RNA/DNA nanoparticles, which include PCT/US2007/013027 (WO2008/039254) (“RNA Nanoparticles and Nanotubes”), filed May 31, 2007, PCT/US2010/038818 (WO 2010/148085) (“RNA Nanoparticles and Nanotubes”), filed June 16, 2010, PCT/US2012/065932 (WO 2013/075132) (“Therapeutic RNA Switches”), filed November 19, 2012, PCT/US2012/065945 (WO 2013/075140) (“Auto-Recognizing Therapeutic RNA/DNA Chimeric Nanoparticles”), filed November 19, 2012, PCT/US2013/058492 (WO 2014/039809) (“Co-Transcriptional Assembly of Modified RNA Nanoparticles”), filed September 6, 2013, PCT/US2014/056007 (WO 2015/042101) (“Multifunctional RNA Nanoparticles and Methods of Use”), filed September 17, 2014, and



PCT/US2015/029553 (WO 2015/171827) (“Triggering RNA Interference with RNA-DNA and DNA-RNA Nanoparticles”), filed May 6, 2015.

In addition, this application incorporates by reference all of Applicant’s prior published scientific journal articles relating to RNA/DNA nanoparticles and the above-indicated prior applications, which include, but is not limited to:

1: Halman JR, Satterwhite E, Roark B, Chandler M, Viard M, Ivanina A, Bindewald E, Kasprzak WK, Panigaj M, Bui MN, Lu JS, Miller J, Khisamutdinov EF, Shapiro BA, Dobrovolskaia MA, Afonin KA. ***Functionally-interdependent shape-switching nanoparticles with controllable properties***. Nucleic Acids Res. 2017 Jan 20. pii: gkx008. doi: 10.1093/nar/gkx008. [Epub ahead of print] PubMed PMID: 28108656.

2: Parlea L, Puri A, Kasprzak W, Bindewald E, Zakrevsky P, Satterwhite E, Joseph K, Afonin KA, Shapiro BA. ***Cellular Delivery of RNA Nanoparticles***. ACS Comb Sci. 2016 Sep 12;18(9):527-47. doi: 10.1021/acscombsci.6b00073. PubMed PMID: 27509068.

3: Parlea L, Bindewald E, Sharan R, Bartlett N, Moriarty D, Oliver J, Afonin KA, Shapiro BA. ***Ring Catalog: A resource for designing self-assembling RNA nanostructures***. Methods. 2016 Jul 1;103:128-37. doi: 10.1016/j.ymeth.2016.04.016. PubMed PMID: 27090005.

4: Afonin KA, Viard M, Tedbury P, Bindewald E, Parlea L, Howington M, Valdman M, Johns-Boehme A, Brainerd C, Freed EO, Shapiro BA. ***The Use of Minimal RNA Toeholds to Trigger the Activation of Multiple Functionalities***. Nano Lett. 2016 Mar 9;16(3):1746-53. doi: 10.1021/acs.nanolett.5b04676. PubMed PMID: 26926382.

5: Afonin KA, Viard M, Kagiampakis I, Case CL, Dobrovolskaia MA, Hofmann J, Vrzak A, Kireeva M, Kasprzak WK, KewalRamani VN, Shapiro BA. ***Triggering of RNA interference with RNA-RNA, RNA-DNA, and DNA-RNA nanoparticles***. ACS Nano. 2015

Jan 27;9(1):251-9. doi: 10.1021/nn504508s. PubMed PMID: 25521794; PubMed Central PMCID: PMC4310632.

6: Afonin KA, Viard M, Koyfman AY, Martins AN, Kasprzak WK, Panigaj M, Desai R, Santhanam A, Grabow WW, Jaeger L, Heldman E, Reiser J, Chiu W, Freed EO, Shapiro BA. ***Multifunctional RNA nanoparticles***. Nano Lett. 2014 Oct 8;14(10):5662-71. doi: 10.1021/nl502385k. PubMed PMID: 25267559; PubMed Central PMCID: PMC4189619.

7: Afonin KA, Kasprzak WK, Bindewald E, Kireeva M, Viard M, Kashlev M, Shapiro BA. ***In silico design and enzymatic synthesis of functional RNA nanoparticles***. Acc Chem Res. 2014 Jun 17;47(6):1731-41. doi: 10.1021/ar400329z. PubMed PMID: 24758371; PubMed Central PMCID: PMC4066900.

8: Afonin KA, Desai R, Viard M, Kireeva ML, Bindewald E, Case CL, Maciag AE, Kasprzak WK, Kim T, Sappe A, Stepler M, Kewalramani VN, Kashlev M, Blumenthal R, Shapiro BA. ***Co-transcriptional production of RNA-DNA hybrids for simultaneous release of multiple split functionalities***. Nucleic Acids Res. 2014 Feb;42(3):2085-97. doi: 10.1093/nar/gkt1001. PubMed PMID: 24194608; PubMed Central PMCID: PMC3919563.

9: Afonin KA, Viard M, Martins AN, Lockett SJ, Maciag AE, Freed EO, Heldman E, Jaeger L, Blumenthal R, Shapiro BA. ***Activation of different split functionalities on re-association of RNA-DNA hybrids***. Nat Nanotechnol. 2013 Apr;8(4):296-304. doi: 10.1038/nnano.2013.44. PubMed PMID: 23542902; PubMed Central PMCID: PMC3618561.

10: Afonin KA, Kireeva M, Grabow WW, Kashlev M, Jaeger L, Shapiro BA. ***Co-transcriptional assembly of chemically modified RNA nanoparticles functionalized with siRNAs***. Nano Lett. 2012 Oct 10;12(10):5192-5. doi: 10.1021/nl302302e. PubMed PMID: 23016824; PubMed Central PMCID: PMC3498980.

11: Afonin KA, Grabow WW, Walker FM, Bindewald E, Dobrovolskaia MA, Shapiro BA, Jaeger L. ***Design and self-assembly of siRNA-functionalized RNA nanoparticles for***

*use in automated nanomedicine*. Nat Protoc. 2011 Dec 1;6(12):2022-34. Doi

10.1038/nprot.2011.418. PubMed PMID: 22134126; PubMed Central PMCID: PMC3498981.

12: Shapiro BA, Bindewald E, Kasprzak W, Yingling Y. *Protocols for the in silico design of RNA nanostructures*. Methods Mol Biol. 2008;474:93-115. doi: 10.1007/978-1-59745-480-3\_7. Review. PubMed PMID: 19031063.

13: Bindewald E, Grunewald C, Boyle B, O'Connor M, Shapiro BA. *Computational strategies for the automated design of RNA nanoscale structures from building blocks using NanoTiler*. J Mol Graph Model. 2008 Oct;27(3):299-308. Doi: 10.1016/j.jmgm.2008.05.004. PubMed PMID: 18838281; PubMed Central PMCID: PMC3744370.

14: Yingling YG, Shapiro BA. *Computational design of an RNA hexagonal nanoring and an RNA nanotube*. Nano Lett. 2007 Aug;7(8):2328-34. PubMed PMID: 17616164.

## **BACKGROUND OF THE INVENTION**

The development of RNA-based nanostructures and their use in a variety of applications, including RNA interference (RNAi) and drug delivery, represents an emerging field of science, technology, and biomedicine often referred to as RNA nanobiology. RNA itself represents a relatively new molecular material for the development of such nanostructures. RNA is a dynamic material because of its natural functionalities, its ability to fold into complex structures, and its capacity to self-assemble. Despite much progress, RNA nanobiology is relatively young and continual improvements are needed in order to fully harness the significant potential of RNA nanostructures in the biomedical arts.

In one aspect, RNA nanobiology provides new possibilities for the diagnosis and treatment of various diseases, such as cancer and viral infections. In particular, RNA nanoparticles are ideal drug delivery devices due to their novel properties and functions and

ability to operate at the same scale as biological entities. Nanoparticles, because of their small size, can penetrate through smaller capillaries and are taken up by cells, which allow efficient drug accumulation at the target sites (Panyam J et al., Fluorescence and electron microscopy probes for cellular and tissue uptake of poly (D, L-lactide-co-glycolide) nanoparticles, *Int J Pharm.* 262:1-11, 2003).

There are several issues that are important for efficient design and drug delivery by nanoparticles, including the efficient attachment of drugs and vectors, controlled drug release, size, toxicity, biodegradability, and activity of the nanoparticle. Moreover, for successful design one needs to understand and control the intermolecular associations, based on natural favorability of interactions and various physical components. Targeted delivery of nanoparticles can be achieved by either passive or active targeting. Active targeting of a therapeutic agent is achieved by conjugating the therapeutic agent or the carrier system to a tissue or cell-specific ligand (Lamprecht et al., Biodegradable nanoparticles for targeted drug delivery in treatment of inflammatory bowel disease, *J Pharmacol Exp Ther.* 299:775-81, 2002). Passive targeting is achieved by coupling the therapeutic agent to a macromolecule that passively reaches the target organ (Monsky W L et al., Augmentation of transvascular transport of macromolecules and nanoparticles in tumors using vascular endothelial growth factor, *Cancer Res.* 59:4129-35, 1999). Drugs encapsulated in nanoparticles or drugs coupled to macromolecules, such as high molecular weight polymers, passively target tumor tissue through the enhanced permeation and retention effect (Maeda H, The enhanced permeability and retention (EPR) effect in tumor vasculature: the key role of tumor- selective macromolecular drug targeting, *Adv Enzyme Regul.* 41:189-207, 2001; Sahoo S K et al., Pegylated zinc protoporphyrin: a water-soluble heme oxygenase inhibitor with tumor-targeting capacity, *Bioconjugate Chem.* 13:1031-8, 2002).

In one application, RNA nanoparticles can be used in connection with RNA interference (RNAi) and the delivery of small interfering RNAs (siRNA). RNAi is a cellular process that regulates gene expression post-transcriptionally. Through the foreign introduction of small interfering RNAs (siRNA), this pathway can be mobilized allowing for the regulation of genes that contribute to the diseased state. While RNA interference continues to hold incredible potential, numerous challenges associated with the application of RNAi technology must be addressed before it can be made into a viable therapy. The most prominent challenges include transporting, targeting several genes inside the same diseased cell, and stabilizing short interfering RNAs (siRNAs). In order to simultaneously target several genes inside the same diseased cell with multiple siRNAs, those siRNAs need to be co-delivered in a controlled fashion, e.g., co-delivery components initially having split or separated functionalities which in the intracellular environment interact to produce one or more desired functionalities, such as siRNA gene silencing. RNA nanotechnology can provide novel technologies for delivery siRNAs that involve the introduction of programmable RNA-based nanoparticles that can be functionalized to work with diverse range of therapeutics.

RNA-based nanoparticles have certain advantages over prior technologies. First, RNA is biocompatible and presents a safe vehicle for the delivery of drugs and therapeutics. Second, RNA has an inherent ability to self-assemble to include various functionalities, including, RNA or DNA aptamers, fluorescent dyes, small molecules, and proteins. In addition, RNA/DNA hybrid systems can be used to split RNA-based functionalities which only become activated when the RNA/DNA hybrid system re-associates to form double stranded RNA. Furthermore, RNA-based nanoparticles made of unmodified nucleotides can be synthesized directly via run-off transcription, making their ease of synthesis and cost of production attractive for scaled-up production. Nevertheless, current RNA-based nanoparticles have certain disadvantages as well, including unwanted immunogenicity, lack of

stability, low and/or irregular production yields, and difficulty of predicting optimal designs. Thus, further improvements in the design and implementation of RNA-based nanoparticles are needed in order to achieve more robust biomedical applications, including for example, in RNA interference. The present invention seeks to solve these problems.

### **SUMMARY OF THE INVENTION**

The present invention relates, in part, to an activatable nanoparticle system comprising at least two interacting cognate nanoparticles having one or more split (and initially inactive) functionalities, wherein said split functionalities are activated as a result of ssRNA-toehold-dependent interaction of the at least two cognate nanoparticles. In certain embodiments, the interacting cognate nanoparticles may each be a RNA/DNA hybrid duplex comprising reverse complement RNA and DNA sequences as between the two nanoparticles, and wherein the RNA strand on each of the nanoparticles further comprises a ssRNA toehold, e.g., 2, 4, 6, 8, 10, 12, or more nucleotides in length. In other embodiments, the cognate nanoparticles may comprise a first nanoparticle having an RNA or DNA “core” nanoscaffold (e.g., a nanoring or nanocube) with functionalized hybrid duplex arms with ssRNA toeholds and a second nanoparticle in the form of a cognate RNA/DNA hybrid duplex with reverse complementary sequences and an ssRNA toehold. The one or more functionalities are initially “split” in an inactive form as between the first and second nanoparticle through their physical separation on the two interacting particles (e.g., separated strands of functional duplex RNA). However, the functionalities become activated upon their reassociation vis-à-vis an ssRNA-toehold-dependent interaction between the nanoparticles. Such split functionalities may include transcriptional activation through the formation of a DNA duplex comprising an (initially split) promoter sequence, the formation of an RNA duplex that functions as a Dicer substrate for siRNA-based gene silencing, the formation of an optical response (e.g., FRET) formed through the joining of initially split optical response moieties (e.g., Alexa 488 and Alexa



546), and reassembly of double-stranded nucleic acid-based aptamers (e.g., Malachite Green isothiocyanate) initially split between the initially inactive interacting cognate nanoparticles.

In certain embodiments, the one or more functionalities are formed as a result of the reassociation of initially separated functionalities that become joined together (e.g., a functional RNA or DNA duplex) and wherein such reassociation is facilitated and/or initiated by way of the reverse complement ssRNA toeholds on each of the interacting nanoparticles.

It was surprisingly and unexpectedly discovered and/or theorized that the herein described activatable nanoparticle system comprising two or more cognate interactable nanoparticles with ssRNA toeholds and split complementary functionalities possessed a number of advantages as compared to corresponding nanoparticles having single strand DNA ("ssDNA") toehold sequences, including greater stability (both chemical and thermal stability), less immunogenicity, smaller size, and greater production yields, e.g., by run-off transcription, and can more readily and reliably be designed through computational methods, as compared to other RNA/DNA hybrid nanoparticles with single stranded DNA toeholds, such as those described in PCT/US2015/029553 (which is incorporated herein by reference).

Without being bound by theory, it is believed that immunogenicity of RNA nanoparticles is a function of the particles' shape and size, among other properties. And, the R/DNA hybrid nanoparticles comprising ssRNA toehold sequences described herein are smaller and more regular shaped than R/DNA nanoparticles comprising ssDNA toehold sequence. Thus, it is theorized that the more regularly shaped and smaller sized RNA nanoparticles described herein would result in RNA-based nanoparticles with less immunogenicity, among other advantages including higher production yield.

Thus, in one aspect, the present invention provides RNA-based nanoparticles comprising ssRNA toeholds which represent an improvement over previously described

RNA-based nanoparticles comprising ssDNA toeholds, e.g., as described in PCT/US2015/029553, which is incorporated herein by reference.

Although RNA nanoparticles comprising ssDNA toeholds were the subject of the inventors' prior application (PCT/US2015/029553), the use of ssRNA toehold sequences was not previously contemplated, described, or used. In part, ssRNA toeholds were not contemplated, described, or used because ssRNA toeholds would not have been a logical modification given that single stranded RNA is well known to form secondary structures. It would have been assumed that such secondary structures would have formed in the single stranded RNA toehold sequences, which would have prevented their functioning in the reassociation process.

Accordingly, in certain aspects, the present invention describes the design and synthesis of various RNA nanoparticles. In preferred embodiments, the nanoparticles of the invention comprise RNA/DNA hybrid systems that utilize single stranded RNA toehold sequence to facilitate their assembly and/or reassociation with cognate RNA/DNA hybrid molecules.

The RNA nanoparticles of the present invention can be designed to self-assemble into predefined size and geometric shapes, in particular rings, squares, cubes, or a three dimensional RNA polyhedral cage any of which can carry multiple components including molecules for specific cell recognition, image detection, and therapeutic treatment, and to encapsulate small therapeutic molecules inside their cages and release them upon being triggered by small ligands. In particular, the RNA nanoparticles of the present invention can be further designed to be spatially addressable by optimizing the location of 3'-tail connectors in the variable stem and thus controlling the positioning of the biotin within the cage. This allows either the encapsulation of proteins inside the cage or their attachment to the outside

forming aggregates of cages. Like proteins and DNA, RNA can potentially lead to stable polyhedral RNA architectures for use as carriers in nano-medicine and synthetic biology.

In other aspects, the RNA nanoparticles of the present invention can be designed to be functionalized with various functional molecules or elements, including multiple short interfering RNAs for combinatorial RNA interference, RNA aptamers, fluorescent dyes, and proteins.

In other aspects, the invention provides RNA/DNA hybrid nanoparticles with conditionally active multiple split functionalities.

In another aspect, the invention features an R/DNA chimeric or hybrid nanoparticle (R/DNA NP) comprising one or more functionalities and including one or more ssRNA toeholds.

Another aspect of the invention provides an R/DNA chimeric nanoparticle (R/DNA NP) having a form of a tube, ring, cube, and the like and having one or more functionalities and including one or more ssRNA toeholds.

In one embodiment, the R/DNA NP possesses one or more RNA-DNA hybrid arm extensions, wherein at least one extension includes an ssRNA toehold. Optionally, one or more of the RNA-DNA hybrid arm extensions is capable of triggered release, formation and/or activation of a dsRNA.

In one embodiment, the functionalities comprise one or more agents. In other embodiments, the agents are selected from one or more of the group consisting of: inhibitory nucleic acids, fluorescent dyes, small molecules, RNA-DNA hybrids with split functionalities, split lipase, split GFP, proteins, therapeutic agents and imaging agents. In a related embodiment, the inhibitory nucleic acids are selected from the group consisting of: siRNAs, RNA or DNA aptamers and ribozymes.

In one embodiment, the one or more agents are the same. In another embodiment, the one or more agents are different.

In one embodiment, the R/DNA nanoparticle comprises at least two chimeric nanoparticles. In another embodiment, the first chimeric nanoparticle comprises a first DNA oligonucleotide and a complementary first RNA oligonucleotide comprising the one or more functionalities, and the second chimeric nanoparticle comprises a second DNA oligonucleotide and a complementary second RNA oligonucleotide comprising the one or more functionalities. In a further embodiment, the first chimeric nanoparticle comprises the sense strand of double stranded (ds) RNA and the second chimeric nanoparticle comprises the antisense strand of dsRNA. In a further embodiment, the first RNA oligonucleotide and the second RNA oligonucleotide comprise ssRNA toehold sequences which extend past the first and second DNA oligonucleotides and are complementary to one another.

In another aspect, the invention provides RNA-DNA and DNA-RNA hybrid nanostructures, e.g., nanocubes, consisting of either RNA or DNA cores (composed of a plurality of strands, e.g., 2, 3, 4, 5, or 6 or more strands of RNA or DNA oligonucleotides) with attached RNA-DNA hybrid duplex "arms" may be used to conditionally activate different functionalities whereby the functional entity or molecule, e.g., Dicer Substrate RNAs, or DS RNAs, RNA aptamers, FRET pair of dyes) is split into two RNA-DNA hybrids, i.e., where a first hybrid is associated with the RNA or DNA nanocube, and a second cognate hybrid is a free RNA-DNA hybrid molecule, both of which are inactive in the hybrid state. In preferred embodiments, the RNA component of the DNA-RNA hybrids further comprise single strand RNA "toeholds" which are complementary as between the nanostructure/nanocube hybrids and freely existing cognate DNA/RNA hybrid molecules and which may interact with one another and trigger the reassociation process when both of the cognate hybrids are present in close proximity. The reassociation process results in strand

swapping to form DNA-DNA and RNA-RNA hybrids, thereby releasing the split functionalities and restoring and/or triggering their function (e.g., Dicer processing to trigger RNA interference). The RNA "toehold" sequences greatly facilitate the reassociation process.

In certain embodiments, the RNA toehold sequences are less than 4 nucleotides in length. In other embodiments, the RNA toehold sequences are 4 or more nucleotides in length. In still other embodiments, the RNA toehold sequences are between 4 and 6 nucleotides, or between 5 and 7 nucleotides, or between 6 and 8 nucleotides, or between 7 and 9 nucleotides, or between 8 and 10 nucleotides, or between 9 and 11 nucleotides, or between 10 and 12 nucleotides, or between 11 and 13 nucleotides, or between 12 and 14 nucleotides, or between 13 and 15 nucleotides, or between 14 and 16 nucleotides, or more than 16 nucleotides. In other embodiments, the single stranded RNA toehold sequences are 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 nucleotides in length.

In one aspect, the present invention is directed to an improvement over the RNA/DNA nanostructures described in the inventors' earlier application, PCT/US2015/029553 (WO2015/171827), which is incorporated herein by reference in its entirety. The improvement in part relates to the use of ssRNA toehold sequences in place the earlier-described ssDNA toehold sequences.

Thus, in one aspect, the present invention relates to using RNA oligonucleotides which self-assemble to form a RNA nanoparticle scaffold, which further comprises RNA oligonucleotide "arms" which are further annealed to cognate DNA oligonucleotides, thereby forming a nanocube structure comprised of an RNA "core" scaffold comprising RNA-DNA hybrid arms with ssRNA toeholds. Such RNA nanocubes having RNA-DNA hybrid arms can then be mixed (i.e., allowed to associate) with cognate free DNA-RNA hybrid molecules (where each DNA oligonucleotide strand of the free DNA-RNA hybrid molecule is antisense to the sequence of the DNA sequence of the RNA-DNA sequences of the hybrid arms of the

nanocube, and vice versa), with such mixing occurring, for example, in solution, in cell culture, following delivery, etc. Such mixing of RNA nanocubes possessing DNA-RNA hybrid arms with free RNA-DNA molecules can promote dissociation and subsequent annealing of arm structures, resulting in RNA nanocubes having RNA-RNA (dsRNA) arms and free dsDNA molecules, thereby activating the innate functionalities of the ds molecules. For example, in certain embodiments, the reassembled dsRNA arms of the RNA nanocubes can be cleaved by Dicer and serve as active RNAi agents (e.g., siRNAs, including, e.g., DsiRNAs), or otherwise serve to activate the RNA interference pathway.

In preferred embodiments, the RNA component of the DNA-RNA hybrids further comprise single strand RNA "toeholds" which are complimentary as between the nanocube hybrids and the freely existing cognate hybrids and which may interact and trigger the reassociation process when both of the cognate hybrids are present in close proximity.

Advantages associated with the inclusion of hybrid arms in an RNA nanocube structure, as compared to entirely RNA nanocubes possessing dsRNA arms, include: reduced immunogenicity, enhanced stability and the functionality of the structures provides the ability to form an initially inactive particle (the RNA scaffold nanocube with hybrid arms) that is then activated for RNAi activity only upon association with a DNA-RNA hybrid molecule that presents strands capable of annealing to corresponding DNA and RNA oligonucleotides of the hybrid arms (where each DNA oligonucleotide strand of the free DNA-RNA hybrid molecule is antisense to the sequence of the DNA sequence of the RNA-DNA sequences of the hybrid arms of the nanocube, and vice versa, therefore driving respective formation of dsDNA and dsRNA duplexes).

Further advantages associated with DNA nanocube scaffolds possessing hybrid arms include: reduced immunogenicity, enhanced stability, a scaffold that can be even more readily labeled (e.g., fluorescently labeled) than an RNA scaffold structure, the functionality of the

structures provides the ability to form an initially inactive particle (the DNA scaffold nanocube with hybrid arms) that then releases activated RNAi agents only upon association with a DNA-RNA hybrid molecule that presents strands capable of annealing to corresponding DNA and RNA oligonucleotides of the hybrid arms (where each DNA oligonucleotide strand of the free DNA-RNA hybrid molecule is antisense to the sequence of the DNA sequence of the RNA-DNA sequences of the hybrid arms of the nanocube, and vice versa, therefore driving respective formation of dsDNA and dsRNA duplexes), the fact that such structures release a free dsRNA (e.g., an RNAi agent, e.g., siRNA or DsiRNA).

Accordingly, in certain aspects, the present invention relates to an activatable nanoparticle system comprising at least two cognate interactable nanoparticles each comprising at least one duplex of DNA and/or RNA each with an ssRNA toehold region, wherein the at least two cognate interactable nanoparticles comprise reverse complement sequences such that when the particles interact, the strands of a first nanoparticle separate and reassociate with the cognate reverse complement strand of the second nanoparticle and wherein the strand reassociation process is facilitated by the presence of the cognate ssRNA toeholds having reverse complement sequences on each of the nanoparticles of the system.

In some embodiments, the activatable nanoparticles comprise DNA nanocubes. The DNA nanocubes may include at least one single-stranded DNA arm, or at least two single-stranded DNA arms, or at least three single-stranded DNA arms, or at least four single-stranded DNA arms, or at least five single-stranded DNA arms, each of which have the capacity to anneal to a cognate or complimentary RNA oligonucleotide. In preferred embodiments, the RNA component of the DNA-RNA hybrids further comprise single strand RNA "toeholds" which are complimentary as between the nanocube hybrids and the freely existing cognate hybrids and which may interact and trigger the reassociation process when both of the cognate hybrids are present in close proximity.



In other embodiments, the DNA nanocubes may include at least one single-stranded RNA arm, or at least two single-stranded RNA arms, or at least three single-stranded RNA arms, or at least four single-stranded RNA arms, or at least five single-stranded RNA arms, each of which have the capacity to anneal to a cognate or complimentary DNA oligonucleotide. In preferred embodiments, the RNA component of the DNA-RNA hybrids further comprise single strand RNA "toeholds" which are complimentary as between the nanocube hybrids and the freely existing cognate hybrids and which may interact and trigger the reassociation process when both of the cognate hybrids are present in close proximity.

In other embodiments, the activatable nanoparticles comprise RNA nanocubes. The RNA nanocubes may include at least one single-stranded RNA arm, or at least two single-stranded RNA arms, or at least three single-stranded RNA arms, or at least four single-stranded RNA arms, or at least five single-stranded RNA arms, each of which have the capacity to anneal to a cognate or complimentary DNA oligonucleotide. In preferred embodiments, the RNA component of the DNA-RNA hybrids further comprise single strand RNA "toeholds" which are complimentary as between the nanocube hybrids and the freely existing cognate hybrids and which may interact and trigger the reassociation process when both of the cognate hybrids are present in close proximity.

In other embodiments, the RNA nanocubes may include at least one single-stranded DNA arm, or at least two single-stranded DNA arms, or at least three single-stranded DNA arms, or at least four single-stranded DNA arms, or at least five single-stranded DNA arms, each of which have the capacity to anneal to a cognate or complimentary RNA oligonucleotide. In preferred embodiments, the RNA component of the DNA-RNA hybrids further comprise single strand RNA "toeholds" which are complimentary as between the nanocube hybrids and the freely existing cognate hybrids and which may interact and trigger the reassociation process when both of the cognate hybrids are present in close proximity.

In one embodiment, the functionalities include one or more sense or antisense strands of at least one RNAi agent.

In another embodiment, the nanocube includes six single-stranded DNA or RNA arms. Optionally, the nanocube includes one, two, three, four, five, six, or more oligonucleotide strands or functional or duplex arms. In preferred embodiments, the nanoparticles comprise six functional duplex arms. In preferred embodiments, each of the duplex arms comprises a ssRNA toehold for interaction with a cognate nanoparticle.

In another embodiment, the RNA-based nanostructures of the invention deliver siRNA which inhibits a target RNA. In a further embodiment, the target RNA is one which produces a therapeutically beneficial result when inhibited. In another further embodiment, the target RNA comprises an RNA that encodes a protein involved in a disease process or a portion thereof. In a further related embodiment of any one of the above aspects, the target RNA encodes an apoptosis inhibitor protein. In another further related embodiment of any one of the above aspects, the target RNA is a pathogenic RNA genome, an RNA transcript derived from the genome of the pathogenic agent, or a portion thereof. In one embodiment, the pathogenic agent is a virus, a bacteria, a fungus, or a parasite. In another embodiment, the target RNA is a viral RNA genome or a portion thereof.

The invention also features a composition comprising an R/DNA NP of any one of the above aspects.

The invention also features a pharmaceutical composition comprising an R/DNA NP of any one of the above aspects.

In one embodiment, the pharmaceutical composition further comprises a pharmaceutically acceptable excipient, carrier, or diluent.

In another embodiment, the pharmaceutical composition is formulated for the treatment of a disease.

In still another embodiment, the pharmaceutical composition is formulated for the treatment of an infection by a pathogenic agent. In another related embodiment, the pathogenic agent is a virus, a bacterium, a fungus, or a parasite.

The invention also features a method of inhibiting or reducing the expression of a target gene in a cell comprising contacting the cell with a therapeutically effective amount of the R/DNA NP of any of the above aspects or embodiments, or the composition of any one of the above aspects or embodiments.

The invention also features a method of killing a pathogen infected cell comprising contacting the cell with a therapeutically effective amount of the R/DNA NP of any one of the above aspects or embodiments or the composition of any one of the above aspects or embodiments.

The invention also features a method of inhibiting replication of a pathogen in a cell comprising contacting the cell with a therapeutically effective amount of the R/DNA NP of any one of the above aspects or embodiments or the composition of any one of the above aspects or embodiments.

In one embodiment, the cell is in a subject.

The invention also features a method of reducing pathogenic burden in a subject comprising administering a therapeutically effective amount of the R/DNA NP of any one of the above aspects or embodiments or the composition of any one of the above aspects or embodiments.

In one embodiment, the subject is at risk of developing a pathogenic infection and/or a tumor.

In another embodiment, the subject is diagnosed with having a pathogenic infection and/or a tumor.

The invention also features a method of treating or preventing a pathogenic infection and/or a tumor in a subject comprising administering a therapeutically effective amount of the R/DNA NP of any one of the above aspects or embodiments or the composition of any one of the above aspects or embodiments.

In one embodiment, the method reduces the pathogenic burden, thereby treating or preventing the pathogenic infection. In another embodiment, the method induces death in infected cell, thereby treating or preventing the pathogenic infection.

In one embodiment, the subject is a mammal. In another embodiment, the subject is a human.

In one embodiment, the pathogen is a virus, bacteria, fungus, or parasite.

In another embodiment of any one of the above aspects or embodiments, the method further comprises contacting the cell with a therapeutically effective amount of a second therapeutic agent or administering a therapeutically effective amount of the second therapeutic agent to the subject.

In one embodiment, the second therapeutic agent treats the pathogenic infection or the symptoms associated with the pathogenic infection.

The invention also features a method of killing a neoplastic cell comprising contacting the cancer cell with a therapeutically effective amount of the of the R/DNA NP of any one of the above aspects or embodiments or the composition of any one of the above aspects or embodiments, thereby killing the neoplastic cell.

The invention also features a method of treating a subject having a neoplasia, the method comprising administering to a subject a therapeutically effective amount of the R/DNA NP of any one of the above aspects or embodiments or the composition of any one of the above aspects or embodiments, thereby treating the subject.

In one embodiment, the neoplastic cell is a cancer cell which is present in a solid tumor.

In another embodiment, the method further comprises contacting the cell with a therapeutically effective amount of a second therapeutic agent or administering a therapeutically effective amount of the second therapeutic agent to the subject.

In one embodiment, the second therapeutic agent is an anti-cancer agent.

The invention also features a kit comprising the R/DNA NP of any one of the above aspects or embodiments or the composition of any one of the above aspects or embodiments.

In one aspect, the kit further comprises a second therapeutic agent.

The invention also features an algorithm for the computational prediction of RNA/DNA hybrid re-association and RNA secondary structures.

In certain aspects, the invention relates to an activatable nanoparticle system comprising one or more split functionalities comprising a first inactive nanoparticle comprising a first set of DNA and/or RNA strands and a first ssRNA toehold and a second inactive nanoparticle comprising a second set of DNA and/or RNA strands and a second ssRNA toehold, wherein the strands of the first inactive nanoparticle are the reverse complements of the strands of the second inactive nanoparticle, wherein the first and second inactive nanoparticles are capable of undergoing reassociation of their strands to produce one or more functionalities, and wherein the reassociation of strands is triggered by the interaction of the first and second ssRNA toeholds.

In other aspects, the invention relates to a method of triggering one or more functionalities in a cell comprising:

(a) administering an effective amount of a first inactive nanoparticle comprising a first set of DNA and/or RNA strands and a first ssRNA toehold;

(b) administering an effective amount of a second inactive nanoparticle comprising a second set of DNA and/or RNA strands and a second ssRNA toehold;

wherein the strands of the first inactive nanoparticle are the reverse complements of the strands of the second inactive nanoparticle,

wherein the first and second inactive nanoparticles are capable of undergoing reassociation of their strands to produce one or more functionalities, and

wherein the reassociation of strands of the first and second nanoparticles is triggered by the interaction of the first and second ssRNA toeholds.

In still other aspects, the invention relates to a method of inhibiting a target gene in a cell comprising:

(a) administering an effective amount of a first inactive nanoparticle comprising a first set of DNA and/or RNA strands and a first ssRNA toehold;

(b) administering an effective amount of a second inactive nanoparticle comprising a second set of DNA and/or RNA strands and a second ssRNA toehold;

wherein the strands of the first inactive nanoparticle are the reverse complements of the strands of the second inactive nanoparticle,

wherein the first and second inactive nanoparticles are capable of undergoing reassociation of their strands to produce one or more functionalities which inhibit a target gene in the cell, and

wherein the reassociation of strands of the first and second nanoparticles is triggered by the interaction of the first and second ssRNA toeholds.

In various embodiments, the one or more split functionalities is selected from the group consisting split transcription, split aptamer, split optical response, and split Dicer substrate.

In other embodiments, the ssRNA toeholds are 2, 4, 6, 8, 10, or 12 nucleotides. In still other embodiments, the ssRNA toeholds are at least 4 nucleotides.

In other embodiments, the ssRNA toeholds impart greater stability (both chemical and thermal stability), less immunogenicity, smaller size, and greater production yields by run-off transcription as compared to nanoparticles with ssDNA toeholds.

In other embodiment still, the first or second nanoparticle is a nanoring, nanotube, or nanocube comprising one or more hybrid duplex arms comprising the first or second ssRNA toehold.

In various embodiments, the first or second nanoparticle is an RNA/DNA duplex comprising the first or second ssRNA toehold.

In still other embodiments, the split Dicer substrate inhibits a target gene.

Other aspects of the invention are described in, or are obvious from, the following disclosure, and are within the ambit of the invention.

### **BRIEF DESCRIPTION OF THE DRAWINGS**

FIG. 1A through 1G show initiation of the re-association of two interacting split-function cognate nanoparticles (each are cognate RNA/DNA hybrids) by RNA toeholds that leads to activation of the resulting functions of RNAi and FRET (optical response signal as a marker of completed strand reassociation).

FIG. 1A is a schematic representation of re-association for cognate interacting nanoparticles (e.g., RNA/DNA hybrids) with 10 nts RNA toeholds. However, in various embodiments, the length of the RNA toeholds may vary, including 2, 4, 6, 8, 10, or 12 or more nucleotides.

FIG. 1B provides a native-PAGE that demonstrates the re-association (1 hour at 37 °C) of fluorescently labeled hybrids with 2, 4, 6, and 8 nts RNA toeholds (at 1  $\mu$ M final).



FIG. 1C: *In vitro* analysis of hybrids with 2, 4, 6, and 8 nts RNA toeholds re-association (but no Dicing) using FRET. Emission of Alexa-488 was measured every 30 sec. Hybrids containing Alexa-546 were added 2 mins after the incubation.

FIG. 1D: FRET experiments: cells were co-transfected with cognate hybrids, with 8 nts RNA toeholds (100 nM final), labeled with Alexa546 and Alexa488 and images were taken on the next day. Image numbers correspond to: (1) - differential interference contrast images, (2) - Alexa488 emission, (3) - Alexa546 emission, (4) - bleed-through corrected FRET image, (5) 3D chart representation of zoomed fragment indicated by a white box of bleed-through corrected FRET image.

FIG. 1E: GFP knockdown assays for GFP expressing human breast cancer cells. Three days after the co-transfection of cells with RNA/DNA hybrids with 2, 4, 6, and 8 nts RNA toeholds, GFP expression was statistically analyzed with flow cytometry experiments. Hybrid concentrations (in nM) used in the silencing experiments are indicated.

FIG. 1F: FRET analysis of concentration dependent re-association of hybrids with 2 nts ssRNA toeholds. The hybrids were incubated at the indicated concentrations for 5 hours in a water bath at 37°C. Upon incubation, they were diluted to the same final concentration of 90 nM final and then subjected to fluorescence analysis. Error bars denote +/- S.E.M. RNA toeholds initiate the re-association of RNA/DNA hybrids that leads to activation of RNAi. Schematic representation of re-association for RNA/DNA hybrids with 8-nts RNA toeholds. Depicted is total staining (with Ethidium Bromide) native-PAGE experiments that demonstrate the re-association of labeled hybrids with 2-, 4-, 6-, and 8-nts RNA toeholds (at 1  $\mu$ M final). The hybrids with 4-nts ssRNA toeholds re-associate partially and hybrids with 2-nts do not re-associate at all.

FIG. 1G: Provides a generalized schematic depicting an activatable nanoparticle system described herein. The top of the schematic depicts a first nanoparticle ("NP1")

corresponding to a first DNA/RNA hybrid having an ssRNA toehold sequence (e.g., 2, 4, 6, 8, 10, or 12 nucleotides). As shown, the first nanoparticle is combined with a second nanoparticle ("NP1"), e.g., inside a target cell, wherein the second nanoparticle is also a hybrid comprising the cognate reverse complement DNA and RNA sequences, and include the reverse complement single stand RNA toehold. During the interaction between the nanoparticles, the reverse complement ssRNA strands hybridize, and then through a process of branch migration, the RNA strands NP1 and NP2 form an RNA duplex. Concomitantly, the DNA strands of NP1 and NP2 form a DNA duplex. The RNA duplex provides for a first functionality, i.e., a Dicer substrate and/or siRNA molecule for gene silencing of a target sequence. The DNA duplex forms an optical marker resulting from the interaction of the split optical response elements (e.g., FRET fluorophores) which are brought together only in the DNA duplex. The optical response can be used to monitor the interaction of NP1 and NP2 and the successful reassociation of their strands. The figure also lists other "exemplary embodiments" for the interacting NP1 and NP2, which can include nanorings and nanocubes comprising split-function hybrids with ssRNA toeholds as NP1, along with cognate split-function hybrid duplexes with ssRNA toeholds as NP2. Split functionalities can include, for example, (a) split siRNA or Dicer substrates, (b) split promoter elements for transcription, (c) split optical response elements (e.g., FRET), (d) split aptamer elements (e.g., Malachite green), among others. The figure also depicts in the lower drawing an activatable nanoparticle system that includes functionalized nanorings decorated with six DNA/RNA hybrids with a ssRNA toehold region, and cognate DNA/RNA hybrids with ssRNA toeholds having the reverse complement sequences. Once combined and allowed to interact, the RNA strand from the cognate hybrids forms a duplex with the RNA strand of the nanoring. Dicing the decorated nanoring produces siRNA molecules.

FIG. 2A through 2C show inhibition of HIV-1 gene expression by an activatable nanoparticle system comprising a pair of cognate RNA/DNA hybrids each with 8 nts RNA toeholds. Hela cells were transfected with an HIV-1 infectious clone, pGluc and nucleic acids as indicated. The control dsRNA targets mRNA of the cellular glutathione S-transferase P1 gene.

FIG. 2A: Transfected Hela cell samples were harvested after 48 h. Cell lysates were probed by western blotting for HIV-1 Gag protein and the constitutively-expressed cellular protein GAPDH.

FIG. 2B: Band intensities of the western blotting of FIG. 2A were measured and Gag expression was calculated as the sum of p55Gag, p41 and CA divided by GAPDH for each sample. These values were expressed relative to HIV-1 in the absence of nucleic acid and the results of four independent experiments were plotted  $\pm$  S.E.M.

FIG. 2C: Samples of culture media were assayed for the RT activity as an indicator of HIV-1 particle release and for the presence of Gaussia luciferase. The RT value was normalized to the Gaussia luciferase value and expressed relative to HIV-1 in the absence of nucleic acids and the results of four independent experiments were plotted. Hybrid concentrations (in nM) used in these gene silencing experiments are indicated. Error bars denote  $\pm$  S.E.M

FIG. 3A shows RNA toeholds initiate the re-association of interacting nanoparticles of an activatable nanoparticle system comprising (a) a nanoring decorated with six RNA/DNA hybrid arms with an ssRNA toehold (e.g., 10 nts), and (b) six cognate RNA/DNA hybrids comprising the reverse complement sequences including the cognate ssRNA toehold (10 nts).

FIG. 3B: Co-transcriptional assembly of functionalized nanorings (NR) was allowed to occur (at 37 °C for 4 hours). Gel purified NR with six hybrids visualized with total staining native-PAGE (left) and re-association (1 hour at 37 °C) of non-labeled NR with six hybrids

(with 8, 6, 4, 2 nts RNA toeholds) traced through the incorporation of fluorescently labeled (with Alexa488 RNA) strands of cognate to NR hybrids. DNA strands are not labeled. The trace amounts of fluorescence at the duplex site of the 8 nt NR lane can be attributed to the excess of hybrid duplexes added to NR.

FIG. 3C: Intracellular re-association of hybrid nanorings (10 nM) and cognate hybrids triggers the GFP silencing. Three days after the co-transfection of cells with nanorings decorated with hybrids and cognate hybrids, GFP silencing was confirmed by fluorescent microscopy.

FIG. 3D: GFP silencing of FIG. 3C was confirmed by statistically analyzed with flow cytometry experiments. Error bars denote  $\pm$  S.E.M.

FIG. 4A through 4D shows *in silico* prediction of RNA/DNA hybrids re-association initiated by ssRNA toeholds.

FIG. 4A: Predicted free energies for individual RNA/DNA hybrids with different lengths of ssRNA toeholds (2, 4, 6, and 8 nts), for their transition states and for their re-associated states (dsRNAs and dsDNAs). Shown are results for concentrations of 0.03, 0.25, 1, 5 and 10  $\mu$ mol/l.

FIG. 4B: Predicted free energies of toehold activation for each transition state model. For high energies of activation the non-reassociated state becomes kinetically trapped. This is the case for 2 and 4 nt toeholds, unless high concentrations are employed. The model of a transition state consists of the non-reassociated RNA/DNA hybrid helices combined with binding of the RNA toeholds. The shown labels indicate toehold lengths.

FIG. 4C: The circular plot depicts intra-strand base pairs as blue arcs and inter-strand base pairs as red arcs. Nanorings are strands A-F and cognate DNAs are strands G-L. Predicted ring scaffold for RNA sequences without toehold arms.

FIG. 4D: The circular plot depicts intra-strand base pairs as blue arcs and inter-strand base pairs as red arcs. Nanorings are strands A-F and cognate DNAs are strands G-L. Predicted ring with hybrid arms and bound cognate DNAs (before re-association).

FIG. 5 shows relative yields of co-transcriptionally assembled RNA nanorings functionalized with RNA-DNA hybrids measured after native-PAGE purification and recovery. Each nanoring was functionalized with six RNA-DNA hybrids of varying ssRNA toehold lengths (8-nts, 6-nts, 4-nts, and 2-nts in length) and is compared with ssDNA toehold.

FIG. 6 shows in silico predictions of re-association of RNA/DNA hybrids initiated by 2-, 4-, 6-, and 8-nts ssRNA toeholds. RNA strands are depicted as red segments; DNA strands are shown in blue. Each base pair corresponds to an arc shown in black.

#### **DETAILED DESCRIPTION OF THE INVENTION**

The present invention relates, in part, to an activatable nanoparticle system comprising at least two interacting cognate nanoparticles having one or more split (and initially inactive) functionalities, wherein said split functionalities are activated as a result of ssRNA-toehold-dependent interaction of the at least two cognate nanoparticles. In certain embodiments, the interacting cognate nanoparticles may each be a RNA/DNA hybrid duplex comprising reverse complement RNA and DNA sequences as between the two nanoparticles, and wherein the RNA strand on each of the nanoparticles further comprises an ssRNA toehold, e.g., 2, 4, 6, 8, 10, 12, or more nucleotides in length. In other embodiments, the cognate nanoparticles may comprise a first nanoparticle having an RNA or DNA “core” nanoscaffold (e.g., a nanoring or nanocube) with functionalized hybrid duplex arms with ssRNA toeholds and a second nanoparticle in the form of a cognate RNA/DNA hybrid duplex with reverse complementary sequences and an ssRNA toehold. The one or more functionalities are initially “split” in an inactive form as between the first and second nanoparticle through their physical separation on the two interacting particles (e.g., separated strands of functional duplex RNA). However,

the functionalities become activated upon their reassociation vis-à-vis an ssRNA-toehold-dependent interaction between the nanoparticles. Such split functionalities may include transcriptional activation through the formation of a DNA duplex comprising an (initially split) promoter sequence, the formation of an RNA duplex that functions as a Dicer substrate for siRNA-based gene silencing, the formation of an optical response (e.g., FRET) formed through the joining of initially split optical response moieties (e.g., Alexa 488 and Alexa 546), and reassembly of double-stranded nucleic acid-based aptamers (e.g., Malachite Green isothiocyanate) initially split between the initially inactive interacting cognate nanoparticles.

Without being bound by theory, it is believed that immunogenicity of RNA nanoparticles is a function of the particles' shape and size, among other properties. And, the R/DNA hybrid nanoparticles comprising ssRNA toehold sequences described herein are smaller and more regular shaped than R/DNA nanoparticles comprising ssDNA toehold sequence. Thus, it is theorized that the more regularly shaped and smaller sized RNA nanoparticles described herein would result in RNA-based nanoparticles with less immunogenicity, among other advantages including higher production yield.

Thus, in one aspect, the present invention provides RNA-based nanoparticles comprising ssRNA toeholds which represent an improvement over previously described RNA-based nanoparticles comprising ssDNA toeholds, e.g., as described in PCT/US2015/029553, which is incorporated herein by reference.

Although RNA nanoparticles comprising ssDNA toeholds were the subject of the inventors' prior application (PCT/US2015/029553), the use of ssRNA toehold sequences was not previously contemplated, described, or used. In part, ssRNA toeholds were not contemplated, described, or used because ssRNA toeholds would not have been a logical modification given that single stranded RNA is well known to form secondary structures. It would have been assumed that such secondary structures would have formed in the single

stranded RNA toehold sequences, which would have prevented their functioning in the reassociation process.

The present invention also relates to the continued development of RNA-based nanoparticles, e.g., siRNA nanoscaffolds, using nanorings, nanocubes, and other three dimensional RNA structures, or RNA/DNA hybrid structures. The RNA-based nanoparticles described herein comprise ssRNA toeholds to promote the exchange of hybrid strands of RNA and DNA as between two different RNA/DNA hybrid nanoparticles (e.g., a hybrid RNA/DNA nanoparticle and a cognate or complimentary RNA/DNA hybrid fragment). In certain embodiments, upon re-association between the hybrid molecules vis-à-vis the action of the RNA toeholds, double stranded RNA/RNA molecules are formed, thereby activating various functionalities which are silent when the RNA is complexed with the DNA prior to reassociation. These functionalized polyvalent RNA/DNA nanoparticles are suitable for therapeutic or diagnostic use in a number of diseases or disorders.

The RNA/DNA nanoparticles described herein have the ability to self-assemble into higher order structures, e.g., a nanoring or nanocube and re-associate to release dsRNA. The nanostructures can also be generated as polyvalent, multifunctional nanoparticles that can bind with different agents to effectively kill a target.

Advantageously, the nanoparticles of the instant invention provide a number of improvements over nanoparticles currently available. For example, the RNA/DNA nanoparticles of the invention do not induce a significant immune response as compared to RNA/DNA nanoparticles having DNA toeholds or to protein nanoparticles currently used. Moreover, the nanoparticles of the invention are smaller than many currently available nanoparticles and therefore allow for increased efficiency of administration. Also, the nanoparticles of the invention with ssRNA toeholds result in higher yield as compared to the nanoparticles comprising ssDNA toeholds. The nanoparticles described herein comprise



multiple RNA subunits each of which has the ability to bind an agent. Moreover, multiple different agents can be present within a single nanoparticle. The multivalency of the RNA-based nanoparticles allows for combining therapeutic (e.g. siRNA/miRNA/drug), targeting (e.g. aptamer and chemical ligand) and detection (e.g. radionucleolide, fluorophore) modules, all in one nanoparticle. The presently disclosed RNA nanostructures provide an effective drug delivery vehicle.

In certain embodiments, the present invention exemplifies how the nanoparticle design can achieve cell-targeting and multifunctional properties by splitting the function of a Dicer Substrate RNA (DS RNA), designed to downregulate the production of green fluorescent protein (GFP) (Rose et al Nucleic acids research 2005, 33, 4140-4156) that is stably expressed in model human breast cancer cells (MDA-MB231/GFP). The use of DS RNAs (as opposed to siRNAs) is required to ensure that once inside the cells, the individual hybrids will not be active in the RNAi pathway (Afonin et al Nucleic acids research 2014, 42, 2085-2097). GFP DS RNAs were split between two RNA-DNA hybrids with the DNA strands being shorter than their corresponding complementary RNAs, thus, providing the ssRNA toeholds for further re-association to form DS RNAs to perform gene silencing. This allows for an additional degree of control over when the therapeutic becomes active. Use of ssRNA toeholds overcomes the need to design specific toeholds as it was needed for ssDNA to prevent undesired interactions and formation of internal secondary structures. ssRNA toeholds addressed several of the challenges remaining in using this technology for a clinical application including but not limited to nanoparticle size, undesired interactions, immunogenicity and multifunctionality.

### **Definitions**

The current invention provides polyvalent R/DNA nanoparticles by using RNA-DNA hybrids and ssRNA toeholds. The polyvalent R/DNA nanoparticles described herein can

further comprise therapeutic, diagnostic and/or delivery agents. Further, the polyvalent R/DNA nanoparticles described herein can be used as drug delivery compositions to treat various diseases or conditions.

The following definitions will be useful in understanding the instant invention.

As used herein, the term "comprising" is intended to mean that the compositions and methods include the recited elements, but do not exclude other elements. "Consisting essentially of", when used to define compositions and methods, shall mean excluding other elements of any essential significance to the combination. Thus, a composition consisting essentially of the elements as defined herein would not exclude trace contaminants from the isolation and purification method and pharmaceutically acceptable carriers, such as phosphate buffered saline, preservatives, and the like. "Consisting of" shall mean excluding more than trace elements of other ingredients and substantial method steps for administering the compositions of this invention. Embodiments defined by each of these transition terms are within the scope of this invention.

As used in the specification and claims, the singular form "a" "an" and "the" include plural references unless the context clearly dictates otherwise.

Ranges provided herein are understood to be shorthand for all of the values within the range. For example, a range of 1 to 50 is understood to include any number, combination of numbers, or sub-range from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, and 50.

Unless specifically stated or obvious from context, as used herein, the term "or" is understood to be inclusive.

The recitation of a listing of chemical groups in any definition of a variable herein includes definitions of that variable as any single group or combination of listed groups. The

recitation of an embodiment for a variable or aspect herein includes that embodiment as any single embodiment or in combination with any other embodiments or portions thereof.

Any compositions or methods provided herein can be combined with one or more of any of the other compositions and methods provided herein.

As used herein, the term "administering" is meant to refer to a means of providing the composition to the subject in a manner that results in the composition being inside the subject's body. Such an administration can be by any route including, without limitation, subcutaneous, intradermal, intravenous, intra-arterial, intraperitoneal, and intramuscular.

As used herein, the term "functionalities" refers to substances which are capable of being contained in, or attached, to the nanoparticle. In exemplary embodiments, functionality is an agent. Exemplary agents include, for example, prodrugs, diagnostic agents, imaging agents, therapeutic agents, chemotherapeutic agents, pharmaceutical agents, drugs, synthetic organic molecules, proteins, peptides, vitamins, and steroids, siRNAs, RNA or DNA aptamers, fluorescent dyes, small molecules, RNA-DNA hybrids with split functionalities, split lipase, split GFP, split Dicer Substrate (DS), FRET (fluorescence resonance energy transfer) pairs, and proteins.

As used herein, the term "split functionalities" refers to combinable subunits of a functional unit which do not have function in isolation, but which gain function when combined. For instance, the activatable nanoparticle systems of the invention may comprise at least two interactable cognate nanoparticles with one or more split functionalities. The interactable nanoparticles of the system have no function on their own in an initial state, but when combined, allowed to interact (via the ssRNA toehold interaction), and the individual cognate and complementary strands of the interacting nanoparticles allowed to reassociate, the resulting newly formed or reassociated nanoparticles become functionalized. Such split functionalities may include transcriptional activation through the formation of a DNA duplex

comprising an (initially split) promoter sequence, the formation of an RNA duplex that functions as a Dicer substrate for siRNA-based gene silencing, the formation of an optical response (e.g., FRET) formed through the joining of initially split optical response moieties (e.g., Alexa 488 and Alexa 546), and reassembly of double-stranded nucleic acid-based aptamers (e.g., Malachite Green isothiocyanate) initially split between the initially inactive interacting cognate nanoparticles.

As used herein, an “aptamer” is an oligonucleotide that is able to specifically bind an analyte of interest other than by base pair hybridization. Aptamers typically comprise DNA or RNA or a mixture of DNA and RNA. Aptamers may be naturally occurring or made by synthetic or recombinant means. The aptamers are typically single stranded, but may also be double stranded or triple stranded. They may comprise naturally occurring nucleotides, nucleotides that have been modified in some way, such as by chemical modification, and unnatural bases, for example 2-aminopurine. See, for example, U.S. Pat. No. 5,840,867. The aptamers may be chemically modified, for example, by the addition of a label, such as a fluorophore, or a by the addition of a molecule that allows the aptamer to be crosslinked to a molecule to which it is bound. Aptamers are of the same “type” if they have the same sequence or are capable of specific binding to the same molecule. The length of the aptamer will vary, but is typically less than about 100 nucleotides. An example of an aptamer may include Malachite green.

As used herein, the term “therapeutic agent” is meant to refer to an agent that is capable of exerting an effect on a target, in vitro or in vivo.

As used herein, the term “chemotherapeutic agent” is meant to include a compound or molecule that can be used to treat or prevent a cancer. A “chemotherapeutic agent ” is meant to include acivicin; aclarubicin; acodazole hydrochloride; acronine; adozelesin; aldesleukin; altretamine; ambomycin; ametantrone acetate; aminoglutethimide; amsacrine; anastrozole;

anthramycin; asparaginase; asperlin; azacitidine; azetepa; azotomycin; batimastat; benzodepa;  
bicalutamide; bisantrene hydrochloride; bisnafide dimesylate; bizelesin; bleomycin sulfate;  
brequinar sodium; broprimine; busulfan; cactinomycin; calusterone; caracemide; carbetimer;  
carboplatin; carmustine; carubicin hydrochloride; carzelesin; cedefingol; chlorambucil;  
cirolemycin; cisplatin; cladribine; crisnatol mesylate; cyclophosphamide; cytarabine;  
dacarbazine; dactinomycin; daunorubicin hydrochloride; decitabine; dexormaplatin;  
dezaguanine; dezaguanine mesylate; diaziquone; docetaxel; doxorubicin; doxorubicin  
hydrochloride; droloxifene; droloxifene citrate; dromostanolone propionate; duazomycin;  
edatrexate; eflornithine hydrochloride; elsamitrucin; enloplatin; enpromate; epipropidine;  
epirubicin hydrochloride; erbulozole; esorubicin hydrochloride; estramustine; estramustine  
phosphate sodium; etanidazole; etoposide; etoposide phosphate; etoprine; fadrozole  
hydrochloride; fazarabine; fenretinide; floxuridine; fludarabine phosphate; fluorouracil;  
flurocitabine; fosquidone; fostriecin sodium; gemcitabine; gemcitabine hydrochloride;  
hydroxyurea; idarubicin hydrochloride; ifosfamide; ilmofofosine; interleukin II (including  
recombinant interleukin II, or rIL2), interferon alfa-2a; interferon alfa-2b; interferon alfa-n1;  
interferon alfa-n3; interferon beta-I a; interferon gamma-I b; iproplatin; irinotecan  
hydrochloride; lanreotide acetate; letrozole; leuprolide acetate; liarozole hydrochloride;  
lometrexol sodium; lomustine; losoxantrone hydrochloride; masoprocol; maytansine;  
mechlorethamine, mechlorethamine oxide hydrochloride rethamine hydrochloride; megestrol  
acetate; melengestrol acetate; melphalan; menogaril; mercaptopurine; methotrexate;  
methotrexate sodium; metoprine; meturedopa; mitindomide; mitocarcin; mitocromin;  
mitogillin; mitomalcin; mitomycin; mitosper; mitotane; mitoxantrone hydrochloride;  
mycophenolic acid; nocodazole; nogalamycin; ormaplatin; oxisuran; paclitaxel; pegaspargase;  
peliomycin; pentamustine; peplomycin sulfate; perfosfamide; pipobroman; piposulfan;  
piroxantrone hydrochloride; plicamycin; plomestane; porfimer sodium; porfiromycin;



prednimustine; procarbazine hydrochloride; puromycin; puromycin hydrochloride;  
pyrazofurin; riboprime; rogletimide; safingol; safingol hydrochloride; semustine; simtrazene;  
sparfosate sodium; sparsomycin; spirogermanium hydrochloride; spiromustine; spiroplatin;  
streptonigrin; streptozocin; sulofenur; talisomycin; tecogalan sodium; tegafur; teloxantrone  
hydrochloride; temoporfin; teniposide; teroxirone; testolactone; thiamiprine; thioguanine;  
thiotepa; tiazofurin; tirapazamine; toremifene citrate; trestolone acetate; tricirbine phosphate;  
trimetrexate; trimetrexate glucuronate; triptorelin; tubulozole hydrochloride; uracil mustard;  
uredepa; vapreotide; verteporfin; vinblastine sulfate; vincristine sulfate; vindesine; vindesine  
sulfate; vinepidine sulfate; vinglycinate sulfate; vinleurosine sulfate; vinorelbine tartrate;  
vinrosidine sulfate; vinzolidine sulfate; vorozole; zeniplatin; zinostatin; zorubicin  
hydrochloride, improsulfan, benzodepa, carboquone, triethylenemelamine,  
triethylenephosphoramidate, triethylenethiophosphoramidate, trimethylolomelamine,  
chlornaphazine, novembichin, phenesterine, trofosfamide, estermustine, chlorozotocin,  
gemzar, nimustine, ranimustine, dacarbazine, mannomustine, mitobronitol, aclacinomycins,  
actinomycin F(1), azaserine, bleomycin, carubicin, carzinophilin, chromomycin,  
daunorubicin, daunomycin, 6-diazo-5-oxo-1-norleucine, doxorubicin, olivomycin, plicamycin,  
porfiromycin, puromycin, tubercidin, zorubicin, denopterin, pteropterin, 6-mercaptopurine,  
ancitabine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, enocitabine, pulmozyme,  
aceglatone, aldophosphamide glycoside, bestabucil, defofamide, demecolcine, elfornithine,  
elliptinium acetate, etoglucid, flutamide, hydroxyurea, lentinan, phenamet, podophyllinic acid,  
2-ethylhydrazide, razoxane, spirogermanium, tamoxifen, taxotere, tenuazonic acid,  
triaziquone, 2,2',2''-trichlorotriethylamine, urethan, vinblastine, vincristine, vindesine and  
related agents. 20-epi-1,25 dihydroxyvitamin D3; 5-ethynyluracil; abiraterone; aclarubicin;  
acylfulvene; adecypenol; adozelesin; aldesleukin; ALL-TK antagonists; altretamine;  
ambamustine; amidox; amifostine; aminolevulinic acid; amrubicin; amsacrine; anagrelide;

anastrozole; andrographolide; angiogenesis inhibitors; antagonist D; antagonist G; antarelix;  
anti-dorsalizing morphogenetic protein-1; antiandrogen, prostatic carcinoma; antiestrogen;  
antineoplaston; antisense oligonucleotides; aphidicolin glycinate; apoptosis gene modulators;  
apoptosis regulators; apurinic acid; ara-CDP-DL-PTBA; arginine deaminase; asulacrine;  
atamestane; atrimustine; axinastatin 1; axinastatin 2; axinastatin 3; azasetron; azatoxin;  
azatyrosine; baccatin III derivatives; balanol; batimastat; BCR/ABL antagonists;  
benzochlorins; benzoylstaurosporine; beta lactam derivatives; beta-alethine; betaclamycin B;  
betulinic acid; bFGF inhibitor; bicalutamide; bisantrene; bisaziridinylspermine; bisnafide;  
bistratene A; bizelesin; breflata; broprimine; budotitane; buthionine sulfoximine; calcipotriol;  
calphostin C; camptothecin derivatives; canarypox IL-2; capecitabine; carboxamide-amino-  
triazole; carboxyamidotriazole; CaRest M3; CARN 700; cartilage derived inhibitor;  
carzelesin; casein kinase inhibitors (ICOS); castanospermine; cecropin B; cetorelix; chlorlins;  
chloroquinoxaline sulfonamide; cicaprost; cisporphyrin; cladribine; clomifene analogues;  
clotrimazole; collismycin A; collismycin B; combretastatin A4; combretastatin analogue;  
conagenin; crambescidin 816; crisnatol; cryptophycin 8; cryptophycin A derivatives; curacin  
A; cyclopentantraquinones; cycloplatam; cypemycin; cytarabine ocfosphate; cytolytic factor;  
cytostatin; dacliximab; decitabine; dehydridemnin B; deslorelin; dexamethasone;  
dexifosfamide; dexrazoxane; dexverapamil; diaziquone; didemnin B; didox;  
diethylnorspermine; dihydro-5-azacytidine; dihydrotaxol, 9; dioxamycin; diphenyl  
spiromustine; docetaxel; docosanol; dolasetron; doxifluridine; droloxifene; dronabinol;  
duocarmycin SA; ebselen; ecomustine; edelfosine; edrecolomab; eflornithine; elemene;  
emitefur; epirubicin; epristeride; estramustine analogue; estrogen agonists; estrogen  
antagonists; etanidazole; etoposide phosphate; exemestane; fadrozole; fazarabine; fenretinide;  
filgrastim; finasteride; flavopiridol; flezelastine; fluasterone; fludarabine; fluorodaunorubicin  
hydrochloride; forfenimex; formestane; fostriecin; fotemustine; gadolinium texaphyrin;



gallium nitrate; galocitabine; ganirelix; gelatinase inhibitors; gemcitabine; glutathione inhibitors; hepsulfam; heregulin; hexamethylene bisacetamide; hypericin; ibandronic acid; idarubicin; idoxifene; idramantone; ilmofofosine; ilomastat; imidazoacridones; imiquimod; immunostimulant peptides; insulin-like growth factor-1 receptor inhibitor; interferon agonists; interferons; interleukins; iobenguane; iododoxorubicin; ipomeanol, 4-; iroplact; irsogladine; isobengazole; isohomohalicondrin B; itasetron; jasplakinolide; kahalalide F; lamellarin-N triacetate; lanreotide; leinamycin; lenograstim; lentinan sulfate; leptolstatin; letrozole; leukemia inhibiting factor; leukocyte alpha interferon; leuprolide+estrogen+progesterone; leuprorelin; levamisole; liarozole; linear polyamine analogue; lipophilic disaccharide peptide; lipophilic platinum compounds; lissoclinamide 7; lobaplatin; lombricine; lometrexol; lonidamine; losoxantrone; lovastatin; loxoribine; lurtotecan; lutetium texaphyrin; lysofylline; lytic peptides; maitansine; mannostatin A; marimastat; masoprocol; maspin; matrilysin inhibitors; matrix metalloproteinase inhibitors; menogaril; merbarone; meterelin; methioninase; metoclopramide; MIF inhibitor; mifepristone; miltefosine; mirimostim; mismatched double stranded RNA; mitoguazone; mitolactol; mitomycin analogues; mitonafide; mitotoxin fibroblast growth factor-saporin; mitoxantrone; mofarotene; molgramostim; monoclonal antibody, human chorionic gonadotrophin; monophosphoryl lipid A+myobacterium cell wall sk; mopidamol; multiple drug resistance gene inhibitor; multiple tumor suppressor 1-based therapy; mustard anticancer agent; mycaperoxide B; mycobacterial cell wall extract; myriaporone; N-acetyldinaline; N-substituted benzamides; nafarelin; nagrestip; naloxone+pentazocine; napavin; naphterpin; nartograstim; nedaplatin; nemorubicin; neridronic acid; neutral endopeptidase; nilutamide; nisamycin; nitric oxide modulators; nitroxide antioxidant; nitrullyn; O6-benzylguanine; octreotide; okicenone; oligonucleotides; onapristone; ondansetron; ondansetron; oracin; oral cytokine inducer; ormaplatin; osaterone; oxaliplatin; oxaunomycin; taxel; taxel analogues; taxel derivatives; palauamine;

palmitoylrhizoxin; pamidronic acid; panaxytriol; panomifene; parabactin; pazelliptine; pegaspargase; peldesine; pentosan polysulfate sodium; pentostatin; pentrozone; perflubron; perfosfamide; perillyl alcohol; phenazinomycin; phenylacetate; phosphatase inhibitors; picibanil; pilocarpine hydrochloride; pirarubicin; piritrexim; placetin A; placetin B; plasminogen activator inhibitor; platinum complex; platinum compounds; platinum-triamine complex; porfimer sodium; porfiromycin; prednisone; propyl bis-acridone; prostaglandin J2; proteasome inhibitors; protein A-based immune modulator; protein kinase C inhibitor; protein kinase C inhibitors, microalgal; protein tyrosine phosphatase inhibitors; purine nucleoside phosphorylase inhibitors; purpurins; pyrazoloacridine; pyridoxylated hemoglobin polyoxyethylene conjugate; raf antagonists; raltitrexed; ramosetron; ras farnesyl protein transferase inhibitors; ras inhibitors; ras-GAP inhibitor; retelliptine demethylated; rhenium Re 186 etidronate; rhizoxin; ribozymes; RII retinamide; rogletimide; rohitukine; romurtide; roquinimex; rubiginone B1; ruboxyl; safingol; saintopin; SarCNU; sarcophytol A; sargramostim; Sdi 1 mimetics; semustine; senescence derived inhibitor 1; sense oligonucleotides; signal transduction inhibitors; signal transduction modulators; single chain antigen binding protein; sizofiran; sobuzoxane; sodium borocaptate; sodium phenylacetate; solverol; somatomedin binding protein; sonermin; sparfosic acid; spicamycin D; spiromustine; splenopentin; spongistatin 1; squalamine; stem cell inhibitor; stem-cell division inhibitors; stipiamide; stromelysin inhibitors; sulfinosine; superactive vasoactive intestinal peptide antagonist; suradista; suramin; swainsonine; synthetic glycosaminoglycans; tallimustine; tamoxifen methiodide; tauromustine; tazarotene; tecogalan sodium; tegafur; tellurapyrylium; telomerase inhibitors; temoporfin; temozolomide; teniposide; tetrachlorodecaoxide; tetrazomine; thaliblastine; thiocoraline; thrombopoietin; thrombopoietin mimetic; thymalfasin; thymopoietin receptor agonist; thymotrnan; thyroid stimulating hormone; tin ethyl etiopurpurin; tirapazamine; titanocene bichloride; topsentin; toremifene;

totipotent stem cell factor; translation inhibitors; tretinoin; triacetyluridine; tricyribine; trimetrexate; triptorelin; tropisetron; turosteride; tyrosine kinase inhibitors; tyrphostins; UBC inhibitors; ubenimex; urogenital sinus-derived growth inhibitory factor; urokinase receptor antagonists; vapreotide; variolin B; vector system, erythrocyte gene therapy; velaresol; veramine; verdins; verteporfin; vinorelbine; vinxaltine; vitaxin; vorozole; zanoterone; zeniplatin; zilascorb; and zinostatin stimalamer. Preferred additional anti-cancer drugs are 5-fluorouracil and leucovorin. Additional cancer therapeutics include monoclonal antibodies such as rituximab, trastuzumab and cetuximab.

As used herein, the term "effective amount" refers to that amount of a therapeutic agent alone that produces the desired effect (such as treatment of a medical condition such as a disease or the like, or alleviation of a symptom such as pain) in a patient. In some aspects, the phrase refers to an amount of therapeutic agent that, when incorporated into a composition of the invention, provides a preventative effect sufficient to prevent or protect an individual from future medical risk associated with a particular disease or disorder. A physician or veterinarian of ordinary skill can readily determine and prescribe the effective amount of the bioactive agent required to treat and/or prevent the progress of the condition.

As used herein, the term "cancer" is used to mean a condition in which a cell in a subject's body undergoes abnormal, uncontrolled proliferation. Thus, "cancer" is a cell-proliferative disorder. Examples of cancers include, without limitation, leukemias (e.g., acute leukemia, acute lymphocytic leukemia, acute myelocytic leukemia, acute myeloblastic leukemia, acute promyelocytic leukemia, acute myelomonocytic leukemia, acute monocytic leukemia, acute erythroleukemia, chronic leukemia, chronic myelocytic leukemia, chronic lymphocytic leukemia), polycythemia vera, lymphoma (Hodgkin's disease, non-Hodgkin's disease), Waldenstrom's macroglobulinemia, heavy chain disease, and solid tumors such as sarcomas and carcinomas (e.g., fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma,

osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilm's tumor, cervical cancer, uterine cancer, testicular cancer, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, schwannoma, meningioma, melanoma, neuroblastoma, and retinoblastoma). Lymphoproliferative disorders are also considered to be proliferative diseases.

The terms "cancer," "neoplasm," and "tumor," are used interchangeably and in either the singular or plural form, refer to cells that have undergone a malignant transformation that makes them pathological to the host organism. By "neoplastic cell" is meant a cell that is a component of a neoplasia.

As used herein, a "composition" refers to the combination of an active agent (e.g., a polyvalent RNA nanoparticle). The composition additionally can comprise a pharmaceutically acceptable carrier or excipient and/or one or more therapeutic agents for use *in vitro* or *in vivo*. A composition may also refer to an activatable nanoparticle system described herein, which may comprise at least two different initially inactive nanoparticles, which when combined are allowed to interact via complementary ssRNA toeholds on each particle, and subsequently are allowed to reassociate into new functional nanoparticles, e.g., a

functional duplex RNA Dicer substrate, or a functional DNA duplex bearing partnered optical response elements (e.g., FRET).

As used herein, the term “conjugated” is understood as attached, linked, or otherwise present on a nanoparticle.

As used herein, “disease” is meant to refer to any condition or disorder that damages or interferes with the normal function of a cell, tissue, or organ.

As used herein, “effective amount” is meant to refer to the amount of a required to ameliorate the symptoms of a disease relative to an untreated patient. The effective amount of active compound(s) used to practice the present invention for therapeutic treatment of a disease varies depending upon the manner of administration, the age, body weight, and general health of the subject. Ultimately, the attending physician or veterinarian will decide the appropriate amount and dosage regimen. Such amount is referred to as an “effective” amount.

The invention provides a number of targets that are useful for the development of highly specific drugs to treat or a disorder characterized by the methods delineated herein. In addition, the methods of the invention provide a facile means to identify therapies that are safe for use in subjects. In addition, the methods of the invention provide a route for analyzing virtually any number of compounds for effects on a disease described herein with high-volume throughput, high sensitivity, and low complexity.

As used herein, “inhibits neoplasia” is meant decreases the propensity of a cell to develop into neoplasia or slows, decreases, or stabilizes the growth or proliferation of a neoplasia.

As used herein, “inhibitory nucleic acid” is meant a double-stranded RNA, siRNA, shRNA, or antisense RNA, or a portion thereof, or a mimetic thereof, that when administered to a mammalian cell results in a decrease (e.g., by 10%, 25%, 50%, 75%, or even 90-100%) in



the expression of a target gene. Typically, a nucleic acid inhibitor comprises at least a portion of a target nucleic acid molecule, or an ortholog thereof, or comprises at least a portion of the complementary strand of a target nucleic acid molecule. For example, an inhibitory nucleic acid molecule comprises at least a portion of any or all of the nucleic acids delineated herein.

As used herein, "kits" are understood to contain at least the non-standard laboratory reagents of the invention and one or more non-standard laboratory reagents for use in the methods of the invention.

As used herein, the term "nanoparticle" is meant to refer to a particle between 10 nm and 200 nm in size. A nanoparticle according to the invention comprises a ribonucleic acid (RNA). The RNA can be obtained from any source, for example bacteriophages phi 29, HIV, *Drosophila*, the ribosome, or be a synthetic RNA.

The term "obtaining" is understood herein as manufacturing, purchasing, or otherwise coming into possession of.

The term "oligonucleotide" as used herein includes linear oligomers of nucleotides or analogs thereof, including deoxyribonucleosides, ribonucleosides, and the like. Typically, oligonucleotides range in size from a few monomeric units, e.g., 3-4, to several hundreds of monomeric units. Oligonucleotides can have inhibitory activity or stimulatory activity.

As used herein, the term "pharmaceutically acceptable carrier" encompasses any of the standard pharmaceutical carriers, such as a phosphate buffered saline solution, water, and emulsions, such as an oil/water or water/oil emulsion, and various types of wetting agents. The compositions also can include stabilizers and preservatives. For examples of carriers, stabilizers and adjuvants, see Martin *Remington's Pharm. Sci.*, 15th Ed. (Mack Publ. Co., Easton (1975)).

The term "subject" is intended to include organisms needing treatment. Examples of subjects include mammals, e.g., humans, dogs, cows, horses, pigs, sheep, goats, cats, mice,

rabbits, rats, and transgenic non-human animals. In certain embodiments, the subject is a human.

The term “toehold” refers to nucleation site of a domain comprising a nucleic acid sequence designed to initiate hybridization of the domain with a complementary nucleic acid sequence.

The term “ssRNA toehold” refers to nucleation site of a domain comprising a ribonucleic acid sequence designed to initiate hybridization of the domain with a complementary nucleic acid sequence. In certain embodiments, the activatable nanoparticle systems described herein comprises at least two interactable and cognate nanoparticles with reverse complement sequences which interact and reassociate (i.e., swapping of complementary strands or reverse complement strands), wherein said interaction and strand swapping begins with an initiating interaction between the ssRNA toeholds on each of the interacting nanoparticles. The ssRNA toeholds can be of any suitable length, including 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 nucleotides, or 2, 4, 6, 8, 10, 12 nucleotides, or 2, 4, 6, or 8 nucleotides, or in some embodiments, 2, 4, or 6 nucleotides. In certain embodiments, the ssRNA toeholds have a minimal length of 2 nucleotides. In other embodiments, the ssRNA toeholds have a minimal length of 3 nucleotides. In still embodiments, the ssRNA toeholds have a minimal length of 4 nucleotides. In yet other embodiments, the ssRNA toeholds have a minimal length of 6 nucleotides. In some embodiments, the ssRNA toeholds have a minimal length of 8 nucleotides. In still other embodiments, the ssRNA toeholds have a minimal length of 10 nucleotides. In other embodiments, the ssRNA toeholds have a minimal length of 12 nucleotides.

As used herein, the term “therapeutic agent” includes a drug and means a molecule, group of molecules, complex or substance administered to an organism for diagnostic, therapeutic, preventative medical, or veterinary purposes. This term includes externally and



internally administered topical, localized and systemic human and animal pharmaceuticals, treatments, remedies, nutraceuticals, cosmeceuticals, biologicals, devices, diagnostics and contraceptives, including preparations useful in clinical screening, prevention, prophylaxis, healing, wellness, detection, imaging, diagnosis, therapy, surgery, monitoring, cosmetics, prosthetics, forensics and the like. This term may also be used in reference to agriceutical, workplace, military, industrial and environmental therapeutics or remedies comprising selected molecules or selected nucleic acid sequences capable of recognizing cellular receptors, membrane receptors, hormone receptors, therapeutic receptors, microbes, viruses or selected targets comprising or capable of contacting plants, animals and/or humans. This term can also specifically include nucleic acids and compounds comprising nucleic acids that produce a bioactive effect, for example deoxyribonucleic acid (DNA), ribonucleic acid (RNA), or mixtures or combinations thereof, including, for example, DNA nanoplexes. Pharmaceutically active agents include the herein disclosed categories and specific examples. It is not intended that the category be limited by the specific examples. Those of ordinary skill in the art will recognize also numerous other compounds that fall within the categories and that are useful according to the invention. Examples include a growth factor, e.g., NGF or GDNF, a steroid, a xanthine, a beta-2-agonist bronchodilator, an anti-inflammatory agent, an analgesic agent, a calcium antagonist, an angiotensin-converting enzyme inhibitors, a beta-blocker, a centrally active alpha-agonist, an alpha-1-antagonist, an anticholinergic/antispasmodic agent, a vasopressin analogue, an antiarrhythmic agent, an antiparkinsonian agent, an antiangina/antihypertensive agent, an anticoagulant agent, an antiplatelet agent, a sedative, an anxiolytic agent, a peptidic agent, a biopolymeric agent, an antineoplastic agent, a laxative, an antidiarrheal agent, an antimicrobial agent, an antifungal agent, a vaccine, a protein, or a nucleic acid. In a further aspect, the pharmaceutically active agent can be coumarin, albumin, steroids such as betamethasone, dexamethasone,

methyprednisolone, prednisolone, prednisone, triamcinolone, budesonide, hydrocortisone, and pharmaceutically acceptable hydrocortisone derivatives; xanthines such as theophylline and doxophylline; beta-2-agonist bronchodilators such as salbutamol, fenterol, clenbuterol, bambuterol, salmeterol, fenoterol; antiinflammatory agents, including antiasthmatic anti-inflammatory agents, antiarthritis antiinflammatory agents, and non-steroidal antiinflammatory agents, examples of which include but are not limited to sulfides, mesalamine, budesonide, salazopyrin, diclofenac, pharmaceutically acceptable diclofenac salts, nimesulide, naproxene, acetaminophen, ibuprofen, ketoprofen and piroxicam; analgesic agents such as salicylates; calcium channel blockers such as nifedipine, amlodipine, and nicardipine; angiotensin-converting enzyme inhibitors such as captopril, benazepril hydrochloride, fosinopril sodium, trandolapril, ramipril, lisinopril, enalapril, quinapril hydrochloride, and moexipril hydrochloride; beta-blockers (i.e., beta adrenergic blocking agents) such as sotalol hydrochloride, timolol maleate, esmolol hydrochloride, carteolol, propranolol hydrochloride, betaxolol hydrochloride, penbutolol sulfate, metoprolol tartrate, metoprolol succinate, acebutolol hydrochloride, atenolol, pindolol, and bisoprolol fumarate; centrally active alpha-2-agonists such as clonidine; alpha-1-antagonists such as doxazosin and prazosin; anticholinergic/antispasmodic agents such as dicyclomine hydrochloride, scopolamine hydrobromide, glycopyrrolate, clidinium bromide, flavoxate, and oxybutynin; vasopressin analogues such as vasopressin and desmopressin; antiarrhythmic agents such as quinidine, lidocaine, tocainide hydrochloride, mexiletine hydrochloride, digoxin, verapamil hydrochloride, propafenone hydrochloride, flecainide acetate, procainamide hydrochloride, moricizine hydrochloride, and disopyramide phosphate; antiparkinsonian agents, such as dopamine, L-Dopa/Carbidopa, selegiline, dihydroergocryptine, pergolide, lisuride, apomorphine, and bromocryptine; antiangina agents and antihypertensive agents such as isosorbide mononitrate, isosorbide dinitrate, propranolol, atenolol and verapamil;

anticoagulant and antiplatelet agents such as coumadin, warfarin, acetylsalicylic acid, and ticlopidine; sedatives such as benzodiazapines and barbiturates; ansiolytic agents such as lorazepam, bromazepam, and diazepam; peptidic and biopolymeric agents such as calcitonin, leuprolide and other LHRH agonists, hirudin, cyclosporin, insulin, somatostatin, protirelin, interferon, desmopressin, somatotropin, thymopentin, pidotimod, erythropoietin, interleukins, melatonin, granulocyte/macrophage-CSF, and heparin; antineoplastic agents such as etoposide, etoposide phosphate, cyclophosphamide, methotrexate, 5-fluorouracil, vincristine, doxorubicin, cisplatin, hydroxyurea, leucovorin calcium, tamoxifen, flutamide, asparaginase, altretamine, mitotane, and procarbazine hydrochloride; laxatives such as senna concentrate, casanthranol, bisacodyl, and sodium picosulphate; antidiarrheal agents such as difenoxine hydrochloride, loperamide hydrochloride, furazolidone, diphenoxylate hydrochloride, and microorganisms; vaccines such as bacterial and viral vaccines; antimicrobial agents such as penicillins, cephalosporins, and macrolides, antifungal agents such as imidazolic and triazolic derivatives; and nucleic acids such as DNA sequences encoding for biological proteins, and antisense oligonucleotides.

As used herein, the term "treated," "treating" or "treatment" includes the diminishment or alleviation of at least one symptom associated or caused by the state, disorder or disease being treated. A subject that has been treated can exhibit a partial or total alleviation of symptoms (for example, tumor load), or symptoms can remain static following treatment according to the invention.. The term "treatment" is intended to encompass prophylaxis, therapy and cure.

As used here, the phrase "5' or 3' sticky ends" is meant to refer to the 3' and/ or 5' protruding ends of DNA or RNA that will bond with complementary sequences of bases. In certain embodiments, the RNA motifs have 5' or 3' sticky ends. In certain embodiments, the 5' or 3' sticky ends are located in the middle of a helix. According to the invention, the 5'

and 3' sticky ends can be engineered to be used for self-assembly of the nanorings into an RNA nanotube.

As used here, the phrase "H\_ant" is meant to refer to the hybrid containing antisense strand of DS RNA.

As used here, the phrase "H\_sen" is meant to refer to the hybrid containing sense strand of DS RNA.

Other definitions appear in context throughout the disclosure.

### **RNA, DNA, and Nanoparticle Design**

The activatable nanoparticle systems and split-functional nanoparticles with ssRNA toeholds described herein may comprise DNA, RNA, and hybrids of DNA and RNA. The present invention contemplates a flexible approach to designing interacting nanoparticles for use in the activatable nanoparticle systems described herein. Indeed, any form or type or category of nanoparticle may be used so long as interacting nanoparticles each comprise one or more complementary or cognate duplexes with ssRNA toeholds wherein the duplexes undergo mutual strand reassociation (i.e., swapping of strands as between duplexes) in a manner that is dependent on the initial interaction of the cognate or complementary ssRNA toeholds in each of the interacting nanoparticles (i.e., ssRNA toehold-dependent interaction). Any of the nanoparticles described or contemplated in the inventors' own publicly-available patent applications or publications may be retrofitted with ssRNA toeholds as described herein. Such publications include any of the following, each of which are incorporated herein by reference:

PCT/US2007/013027 (WO2008/039254) ("RNA Nanoparticles and Nanotubes"), filed May 31, 2007, PCT/US2010/038818 (WO 2010/148085) ("RNA Nanoparticles and Nanotubes"), filed June 16, 2010, PCT/US2012/065932 (WO 2013/075132) ("Therapeutic RNA Switches"), filed November 19, 2012, PCT/US2012/065945 (WO 2013/075140) ("Auto-

Recognizing Therapeutic RNA/DNA Chimeric Nanoparticles”), filed November 19, 2012, PCTUS2013/058492 (WO 2014/039809) (“Co-Transcriptional Assembly of Modified RNA Nanoparticles”), filed September 6, 2013, PCT/US2014/056007 (WO 2015/042101) (“Multifunctional RNA Nanoparticles and Methods of Use”), filed September 17, 2014, and PCT/US2015/029553 (WO 2015/171827) (“Triggering RNA Interference with RNA-DNA and DNA-RNA Nanoparticles”), filed May 6, 2015.

Any of the nanoparticles described in the inventors’ own scientific peer-reviewed articles may also be retrofitted with ssRNA toeholds and used in the activatable nanoparticles systems of the invention. The references include:

1: Halman JR, Satterwhite E, Roark B, Chandler M, Viard M, Ivanina A, Bindewald E, Kasprzak WK, Panigaj M, Bui MN, Lu JS, Miller J, Khisamutdinov EF, Shapiro BA, Dobrovolskaia MA, Afonin KA. ***Functionally-interdependent shape-switching nanoparticles with controllable properties***. Nucleic Acids Res. 2017 Jan 20. pii: gkx008. doi: 10.1093/nar/gkx008. [Epub ahead of print] PubMed PMID: 28108656.

2: Parlea L, Puri A, Kasprzak W, Bindewald E, Zakrevsky P, Satterwhite E, Joseph K, Afonin KA, Shapiro BA. ***Cellular Delivery of RNA Nanoparticles***. ACS Comb Sci. 2016 Sep 12;18(9):527-47. doi: 10.1021/acscmbsci.6b00073. PubMed PMID: 27509068.

3: Parlea L, Bindewald E, Sharan R, Bartlett N, Moriarty D, Oliver J, Afonin KA, Shapiro BA. ***Ring Catalog: A resource for designing self-assembling RNA nanostructures***. Methods. 2016 Jul 1;103:128-37. doi: 10.1016/j.ymeth.2016.04.016. PubMed PMID: 27090005.

4: Afonin KA, Viard M, Tedbury P, Bindewald E, Parlea L, Howington M, Valdman M, Johns-Boehme A, Brainerd C, Freed EO, Shapiro BA. ***The Use of Minimal RNA Toeholds to Trigger the Activation of Multiple Functionalities***. Nano Lett. 2016 Mar 9;16(3):1746-53. doi: 10.1021/acs.nanolett.5b04676. PubMed PMID: 26926382.



5: Afonin KA, Viard M, Kagiampakis I, Case CL, Dobrovolskaia MA, Hofmann J, Vrzak A, Kireeva M, Kasprzak WK, KewalRamani VN, Shapiro BA. ***Triggering of RNA interference with RNA-RNA, RNA-DNA, and DNA-RNA nanoparticles***. ACS Nano. 2015 Jan 27;9(1):251-9. doi: 10.1021/nn504508s. PubMed PMID: 25521794; PubMed Central PMCID: PMC4310632.

6: Afonin KA, Viard M, Koyfman AY, Martins AN, Kasprzak WK, Panigaj M, Desai R, Santhanam A, Grabow WW, Jaeger L, Heldman E, Reiser J, Chiu W, Freed EO, Shapiro BA. ***Multifunctional RNA nanoparticles***. Nano Lett. 2014 Oct 8;14(10):5662-71. doi: 10.1021/nl502385k. PubMed PMID: 25267559; PubMed Central PMCID: PMC4189619.

7: Afonin KA, Kasprzak WK, Bindewald E, Kireeva M, Viard M, Kashlev M, Shapiro BA. ***In silico design and enzymatic synthesis of functional RNA nanoparticles***. Acc Chem Res. 2014 Jun 17;47(6):1731-41. doi: 10.1021/ar400329z. PubMed PMID: 24758371; PubMed Central PMCID: PMC4066900.

8: Afonin KA, Desai R, Viard M, Kireeva ML, Bindewald E, Case CL, Maciag AE, Kasprzak WK, Kim T, Sappe A, Stepler M, Kewalramani VN, Kashlev M, Blumenthal R, Shapiro BA. ***Co-transcriptional production of RNA-DNA hybrids for simultaneous release of multiple split functionalities***. Nucleic Acids Res. 2014 Feb;42(3):2085-97. doi: 10.1093/nar/gkt1001. PubMed PMID: 24194608; PubMed Central PMCID: PMC3919563.

9: Afonin KA, Viard M, Martins AN, Lockett SJ, Maciag AE, Freed EO, Heldman E, Jaeger L, Blumenthal R, Shapiro BA. ***Activation of different split functionalities on re-association of RNA-DNA hybrids***. Nat Nanotechnol. 2013 Apr;8(4):296-304. doi: 10.1038/nnano.2013.44. PubMed PMID: 23542902; PubMed Central PMCID: PMC3618561.

10: Afonin KA, Kireeva M, Grabow WW, Kashlev M, Jaeger L, Shapiro BA. ***Co-transcriptional assembly of chemically modified RNA nanoparticles functionalized with***

*siRNAs*. Nano Lett. 2012 Oct 10;12(10):5192-5. doi: 10.1021/nl302302e. PubMed PMID: 23016824; PubMed Central PMCID: PMC3498980.

11: Afonin KA, Grabow WW, Walker FM, Bindewald E, Dobrovolskaia MA, Shapiro BA, Jaeger L. *Design and self-assembly of siRNA-functionalized RNA nanoparticles for use in automated nanomedicine*. Nat Protoc. 2011 Dec 1;6(12):2022-34. Doi: 10.1038/nprot.2011.418. PubMed PMID: 22134126; PubMed Central PMCID: PMC3498981.

12: Shapiro BA, Bindewald E, Kasprzak W, Yingling Y. *Protocols for the in silico design of RNA nanostructures*. Methods Mol Biol. 2008;474:93-115. doi: 10.1007/978-1-59745-480-3\_7. Review. PubMed PMID: 19031063.

13: Bindewald E, Grunewald C, Boyle B, O'Connor M, Shapiro BA. *Computational strategies for the automated design of RNA nanoscale structures from building blocks using NanoTiler*. J Mol Graph Model. 2008 Oct;27(3):299-308. Doi: 10.1016/j.jmglm.2008.05.004. PubMed PMID: 18838281; PubMed Central PMCID: PMC3744370.

14: Yingling YG, Shapiro BA. *Computational design of an RNA hexagonal nanoring and an RNA nanotube*. Nano Lett. 2007 Aug;7(8):2328-34. PubMed PMID: 17616164.

All of these references are incorporated herein by reference in their entireties.

RNA has a number of advantages for nanostructure design. Nanoparticle structures provide a size range that is large enough to avoid the problem of expulsion from the cell, but are small enough to avoid the problems of cell delivery often encountered with larger particles. RNA is the only biopolymer that can carry genetic information and has catalytic properties. RNA can naturally fold into complex motifs, and RNA motifs are capable of self-assembly. RNA has a natural functionality, for instance RNA can function as ribozymes or riboswitches. Further, RNA is advantageous in eliciting a very low immune response.



Moreover, the construction of RNA into ordered, patterned superstructures has a number of desirable characteristics, including the ability to self-assemble in precisely defined ways, the ability to undergo editing and replication, the ability to undergo controlled disassembly. RNA has versatility in function and structure. Functionally, RNA is the only biopolymer that can carry genetic information and that possesses catalytic properties. Structurally, RNA has predictable intra and intermolecular interactions with well-known structural geometry. The RNA strands that consist of adenine (A), guanine (G), cytosine (C), and uridine (U) can naturally, or can be programmed, to self-assemble via complementary base pairing. The helical region of RNA has a well-known nanometer scale structural geometry of 2.86 nm per helical turn with 11 base pairs and a 2.3 nm diameter. The self-assembly of RNA into complex structures can be facilitated via complementary base pairing or inter- and intra-molecular interactions of the different single stranded regions in the RNA, including internal bulges and loop motifs, and single-stranded overhangs or “sticky-ends”. In addition to Watson-Crick base pairing, A, G, C and T can also pair with other, unconventional bases (i.e. non-canonical base-pairing).

The methods of the invention can be used to assemble RNA NPs composed of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125 or more distinct RNA strands.

### **RNA and DNA Synthesis**

The activatable nanoparticles of the invention comprise DNA and RNA molecules which may be made using any suitable and known means. For example, RNA and DNA

molecules used to make the nanoparticles of the invention can be produced recombinantly or synthetically by methods that are routine for one of skill in the art. For example, synthetic RNA molecules can be made as described in US Patent Application Publication No.: 20020161219, or US Patent Nos: 6,469,158, 5,466,586, 5,281,781, or 6,787,305.

Fluorescently labeled RNA molecules were purchased from Integrated DNA Technologies, Inc.

### **RNA Self-Assembly**

The activatable nanoparticles of the invention may rely on the characteristics of RNA self-assembly to form nanoparticles. Small RNA structural motifs can code the precise topology of large molecular architectures. It has been shown that RNA structural motifs participate in a predictable manner to stabilize, position and pack RNA helices without the need of proteins (Chworos A et al., Science 306:2068-2072.2004). RNAI and RNAII are loop structures that interact in what is called a 'kiss' or 'kissing' complex (Lee et al., Structure 6:993-1005.1998). This contact facilitates the pairing of the RNAI and RNAII loops, until the two RNAs form a duplex. As such, the "kissing" interaction between RNAI and RNAII is one means of self-assembly between the RNA building blocks. This self-assembly strategy relies on the particular geometry of bended kissing complexes. The nanorings formed, can be functionalized with siRNA sequences and are capable of being processed by dicer, can be used as siRNA delivery systems (Grabow et al., Nano letters 11:878-887.2011) has potential for developing siRNAs delivery agents.

The self-assembly of nanoparticles from RNA involves cooperative interaction of individual RNA molecules that spontaneously assemble in a predefined manner to form a larger two- or three-dimensional structure. Within the realm of self-assembly two main categories have been described: template and non-template (Lee et al. J Nanosci Nanotechnol. 2005 Dec; 5(12):1964-82). Template assembly involves interaction of RNA molecules under

the influence of specific external sequence, forces, or spatial constraints such as RNA transcription, hybridization, replication, annealing, molding, or replicas. In contrast, non-template assembly involves formation of a larger structure by individual components without the influence of external forces. Examples of non-template assembly are ligation, chemical conjugation, covalent linkage, and loop/loop interaction of RNA, especially the formation of RNA multimeric complexes (Lee et al. 2005, as above).

Previously, RNA has been demonstrated to assemble into nanoparticles of various shapes and sizes. The first RNA nanoparticles were generated using loop-receptor interfaces to form dimeric nanoparticles. The assembly of this H-shaped nanoparticle was mediated by GAAA/Hnt receptor interaction, which is a highly recurrent motif found in group I and group II introns and other ribozymes and riboswitches. This interaction was further used to generate oriented filaments by combining multiple loop-receptor interactions with a four-way junction motif. One of the first examples of RNA nanoparticles that incorporate multiple RNA motifs within its context is the tectosquare, which is composed of four artificial RNA building blocks called tectoRNAs that self-assemble through specific, non-covalent loop-loop interactions called kissing loops (KL) found at the end of each stem. These tectoRNAs were further programmed to self-assemble into complex arrays via 3' sticky tails with controllable topology, directionality and geometry. The first example of a therapeutic RNA nanoparticle was designed from phi-29-encoded packaging motor (pRNA), a natural RNA motif found in bacteriophages. The pRNA dimers were reengineered for targeted delivery of ribozymes to attack the hepatitis B virus by specifically cleaving the virus's poly-A signal. In a subsequent study, the pRNA trimers were functionalized with cell receptor-binding RNA aptamers and were used to deliver siRNAs that target a specific gene for silencing and thus enabling apoptosis in cancer cells.

#### **Nanoparticle Characteristics and Manufacture**

As used herein, the terms “nanoparticle” and “nanoscaffold” are interchangeable. The terms “nanotube,” “nanoring,” and “nanocube” may refer to specific three dimensional forms of the nanoparticles, all of which are contemplated herein. In preferred embodiments, the invention relates to RNA-based nanoparticles (e.g., nanorings or nanocubes) comprising a core structure (e.g., a ring, square, or cube) of DNA or RNA which is functionalized with one or more DNA/RNA hybrid “arms” attached thereto, wherein the RNA component of the hybrid arms comprises a single stranded RNA toehold sequence.

The core structure of any of the activatable nanoparticles of the invention can be in the shape of any two-dimensional shape, such as a ring (e.g., a hexagonal ring), a square, a sheet, or the like. The core structure can also be in the shape of any three-dimensional structure, such as, a cube, a prism, or a tube. In preferred embodiments, the core structure is formed of self-assembly RNA oligonucleotides. Methods for forming various core structures (e.g., nanorings, nanotubes, or nanocubes) have been described in the inventors’ prior applications, for example in PCT/US2007/013027 (“RNA Nanoparticles and Nanotubes”), filed May 31, 2007, PCT/US2010/038818 (“RNA Nanoparticles and Nanotubes”), filed June 16, 2010, PCT/US2012/065932 (“Therapeutic RNA Switches”), filed November 19, 2012, PCT/US2012/065945 (“Auto-Recognizing Therapeutic RNA/DNA Chimeric Nanoparticles”), filed November 19, 2012, PCT/US2013/058492 (“Co-Transcriptional Assembly of Modified RNA Nanoparticles”), filed September 6, 2013, PCT/US2014/056007 (“Multifunctional RNA Nanoparticles and Methods of Use”), filed September 17, 2014, and PCT/US2015/029553 (“Triggering RNA Interference with RNA-DNA and DNA-RNA Nanoparticles”), each of which is incorporated herein by reference.

In certain embodiments, the core portion of the RNA-based nanoparticles of the invention may be modified by attaching one or more DNA/RNA hybrid “arm” that comprises a latent functionality that forms only upon reassociating the DNA/RNA hybrid “arm” with a

cognate free DNA/RNA hybrid molecule to form a functional RNA/RNA duplex. The RNA/RNA duplex could be, for example, an siRNA molecule for triggering RNA interference of a specific target. In addition, the RNA/RNA duplex could comprise FRET pairs, which are activated to produce fluorescence only upon the formation of the RNA/RNA duplex. The hybrid arms preferably comprise an ssRNA toehold sequence for facilitating interaction between two or more cognate nanoparticles described herein.

Methods and sequences for producing a RNA-based nanoparticle having a RNA or DNA core and functionalized with a DNA/RNA hybrid “arm” can be found described, for example, in PCT/US2014/056007 (“Multifunctional RNA Nanoparticles and Methods of Use”), filed September 17, 2014, and PCT/US2015/029553 (“Triggering RNA Interference with RNA-DNA and DNA-RNA Nanoparticles”), which are incorporated herein by reference.

In certain preferred embodiments, the RNA component of the DNA/RNA hybrid “arm” comprises a single stranded RNA “toehold” sequence that extends from the end the hybrid arm by at least 1 additional ribonucleotide. In certain embodiments, the RNA toehold sequences (i.e., the portion of the RNA component of the DNA/RNA hybrid that is single stranded) are less than 4 nucleotides in length. In other embodiments, the RNA toehold sequences are 4 or more nucleotides in length. In still other embodiments, the RNA toehold sequences are between 4 and 6 nucleotides, or between 5 and 7 nucleotides, or between 6 and 8 nucleotides, or between 7 and 9 nucleotides, or between 8 and 10 nucleotides, or between 9 and 11 nucleotides, or between 10 and 12 nucleotides, or between 11 and 13 nucleotides, or between 12 and 14 nucleotides, or between 13 and 15 nucleotides, or between 14 and 16 nucleotides, or more than 16 nucleotides. In other embodiments, the single stranded RNA toehold sequences are 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 nucleotides in length.

Using natural or artificially selected RNA motifs and modules, RNA molecules can be programmed to form a wide variety of compact and stable artificial three-dimensional nanostructures (called RNA NPs; Afonin et al. *Accounts of Chemical Research* 2014, dx.doi.org/10.1021/ar400329z; Afonin et al. *Nat Nanotechnol* 2010, 5, (9), 676-82; Severcan et al. *Nat Chem* 2010, 2, (9), 772-9; Grabow et al. *Nano Lett* 2011, 11, (2), 878-87; Guo et al., *M. Mol Cell* 1998, 2, (1), 149-55) suitable for the broad range of clinical and nanotechnological applications (Afonin et al. *Accounts of Chemical Research* 2014, dx.doi.org/10.1021/ar400329z; Afonin et al. *Nat Protoc* 2011, 6, (12), 2022-34; Guo, P. *Nat Nanotechnol* 2010, 5, (12), 833-42; Shukla et al. *ACS Nano* 2011, 5, (5), 3405-3418; Shu et al. *Rna* 2013, 19, (6), 767-77; Koyfman et al. *J Am Chem Soc* 2005, 127, (34), 11886-7; Shu et al. *Adv Drug Deliv Rev* 2014, 66C, 74-89; Khisamutdinov et al. *ACS Nano* 2014; Hao et al. *Nat Commun* 2014, 5, 3890; Ohno et al. *Nat Nanotechnol* 2011, 6, (2), 116-20; Osada et al. *ACS Nano* 2014; Haque et al. *Nano Today* 2012, 7, (4), 245-257; Tarapore et al. *Mol Ther* 2011, 19, (2), 386-94). Therapeutic nucleic acids, proteins, or small molecules can be individually attached using different techniques (Shu et al. *Adv Drug Deliv Rev* 2014, 66C, 74-89) to programmable RNA toeholds entering the composition of R/DNA NP. The assembly of the monomers will bring the desired functionalities together, thus providing precise control over their topology, composition, and modularity. The use of functional R/DNA NP with ssRNA toeholds *in vivo* will guarantee higher concentration and desired stoichiometry of therapeutic moieties locally.

Herein, new multifunctional R/DNA NPs built based on previously designed RNA nanorings (Grabow et al. *Nano Lett* 2011, 11, (2), 878-87; Yingling and Shapiro. *Nano Lett* 2007, 7, (8), 2328-34) were identified, with the inventions illustrating how this system can be used to address several present challenges associated with RNA NPs including functionalization with different classes of molecules such as multiple siRNAs, aptamers,

proteins, and small molecules. Detailed characterization of the resulting functional RNA NPs *in vitro* (by native-PAGE, DLS, cryo-EM, and fluorescent studies), in various cell cultures and *in vivo* was demonstrated.

As the proof of concept, the function of Dicer Substrate RNA (DS RNA) was split between two R/DNA hybrids. DS RNA was designed to downregulate the production of green fluorescent protein (GFP) (Rose *et al. Nucleic acids research* 2005, 33, 4140-4156) that is stably expressed in model human breast cancer cells (MDA-MB231/GFP). The use of DS RNAs (as opposed to siRNAs) is required to ensure that once inside the cells, the individual hybrids will not be active in the RNAi pathway (Afonin *et al. Nucleic acids research* 2014, 42, 2085-2097.). GFP DS RNAs were split between two RNA-DNA hybrids with the DNA strands being 8-, 6-, 4-, and 2-nts shorter than their corresponding complementary RNAs, thus, providing the ssRNA toeholds for further re-association. A scheme explaining the re-association of new hybrids studied in this work is shown in FIG. 1A.

The incorporation of RNA functionalities such as Dicer Substrate (DS) RNAs (Rose *et al. Nucleic Acids Res* 2005, 33, (13), 4140-56) into the nanoscaffolds presented difficulties in terms of solid state chemical synthesis as RNA components generally cannot exceed ~60 nucleotides in length. This problem was addressed by using small SS RNA toeholds with split functionalities in the cognate hybrids.

Lastly, it has been established herein how the therapeutic functionality of the nanoring can be triggered through the incorporation of multiple agents/functionalities at ssRNA toeholds of RNA-DNA hybrids. This newly developed technique (Afonin *et al. Nat Nanotechnol* 2013, 8, (4), 296-304; Afonin *et al. Acc Chem Res* 2014) involves splitting the different functionalities between cognate RNA-DNA hybrids with further conditional intracellular activation of these functionalities.



In addition to functionalization with multiple different short interfering RNAs for combinatorial RNA interference (e.g. against multiple HIV-1 genes), nanorings of the invention also allow simultaneous embedment of fluorescent dyes, proteins, as well as recently developed RNA-DNA hybrids aimed to conditionally activate multiple split functionalities inside cells.

### **Design**

The general approach used to create RNA nano-particles and nano-materials is to take known RNA structures, cut them into the building blocks, and reengineer single-stranded loops and regions to facilitate the desired self-assembly. The self-assembly of all the above discussed RNA building blocks into nanostructures is mediated by the complementarity of hairpin loops and loop receptors that form non-covalent RNA-RNA interactions. For precise assembly of the RNA building blocks, each of the corresponding complementary loop-loop interactions are uniquely reengineered.

Two main experimental approaches can be used for programmable self-assembly of nucleic acids nanostructures (Jaeger, L.; Chworos, A. *Curr Opin Struct Biol* 2006, 16, (4), 531-43). The first is a single-step assembly, which is commonly used for DNA nanostructures (Chelyapov, N.; Brun, Y.; Gopalkrishnan, M.; Reishus, D.; Shaw, B.; Adleman, L. *J Am Chem Soc* 2004, 126, (43), 13924-5; Mathieu, F.; Liao, S.; Kopatsch, J.; Wang, T.; Mao, C.; Seeman, N. C. *Nano Lett* 2005, 5, (4), 661-5.). The second is a stepwise assembly, which has been commonly described for RNA nanostructures (Chworos, A.; Severcan, I.; Koyfman, A. Y.; Weinkam, P.; Oroudjev, E.; Hansma, H. G.; Jaeger, L. *Science* 2004, 306, (5704), 2068-72). In the single-step assembly approach, all molecules are mixed together followed by the slow cool annealing procedure. This is only possible if the target building block structure is the one that has the highest number of Watson-Crick base pairs and is therefore the most stable. However, it is understood that thermodynamic stability of

different shapes of nanoparticles is also an important consideration, at times more so than Watson base pairing. This approach is, thus, based on the preferential folding of the building blocks at higher temperatures followed by the self-assembly of these building blocks through weaker interactions into final nanostructures at lower temperatures. However, usually there are many other possible structures that are only slightly less stable. In this case, the stepwise approach can be used where the building blocks are separately formed in the first step are then mixed together in the presence of high magnesium ( $Mg^{++}$ ) concentration to form a final nanostructure. This approach is more time consuming and the melting temperatures of the building blocks and the final nanostructure should be well separated.

A number of RNA motifs are available as building blocks, including but not limited to RNA I and/or RNA II motifs, kissing loops, RNA I inverse (RNA Ii) and/ or RNA II inverse (RNA Iii) motifs. As used herein, the term “motif” in reference to a nanoparticle is meant to refer to a double-stranded or single-stranded ribonucleic acid or analog thereof. Individual motifs are joined together into larger particles by attachment to each other. Attachment can occur by non-covalent linking. Numerous high-resolution RNA structures determined by NMR or X-ray crystallography can be separated into building blocks for design of new RNA nanoparticles and nanomaterials. U.S. Application 13/378,985, incorporated by reference in its entirety herein, describes methods of making RNA nanoparticles.

The RNA NPs comprising one or more functionalities according to the invention can be in the shape of a ring, in the shape of a square or in the shape of a triangle; however it is to be understood that other geometries are possible. In certain embodiments, there is a positive relationship between the stability of RNA assemblies and the complexity of the tertiary structures that define the assembly.

**R/DNA hybrid duplexes**

In various embodiments, e.g., FIG. 1G, the interacting nanoparticles described herein comprise one or more functional hybrid duplex “arms.” It is preferred the arms comprise a ssRNA toehold sequence to catalyze or initiate strand reassociation/swapping between the interacting nanoparticles comprising the hybrid duplexes. In certain embodiments, the present invention splits the functionality, e.g., Dicer substrates siRNA duplexes, into two R/DNA hybrids, which upon simultaneous presence inside the same diseased cell will recognize each other through toehold interaction and re-associate releasing active siRNAs. This approach will overcome several challenges associated with the clinical delivery of RNAi, such as intravascular degradation (will be reduced for R/DNA hybrids), tissue specificity (DNA chemistry is more parsimonious than RNA and amenable to chemical modifications with different features for targeting or delivery), pharmacodynamics (fluorescent tags can be activated upon R/DNA hybrid re-association assisting in Förster resonance energy transfer (FRET) imaging of delivery and response). R/DNA hybrids are described in PCT/US2012/065945 and incorporated by reference in its entirety herein.

Using RNA interference (RNAi) as a therapeutic agent it is routinely possible to knock down the expression of target genes in diseased cells. In certain embodiments, the ability of the hybrids with ssRNA toeholds to enter and re-associate inside mammalian cells was assessed (FIG. 1D). The invention features a method for siRNA release where cognate hybrids are co-delivered to the cell either on the same or on two different days. The invention provides for nucleic acids based “smart” nanoparticles for biomedical applications.

To demonstrate the generality and the therapeutic potential of this approach, two additional sets of hybrids were designed against the full-length genomic HIV-1 RNA that also serves as the mRNA coding for the viral structural proteins and enzymes (Berkhout et al. Antiviral research 2011, 92, 7-14; Liu et al., Molecular therapy : the journal of the American

Society of Gene Therapy 2009, 17, 1712-1723; Low et al. Molecular therapy : the journal of the American Society of Gene Therapy 2012, 20, 820-828; Olivier et al. Molecular therapy : the journal of the American Society of Gene Therapy 2008, 16, 557-564). Gag and LDR hybrids targeted the sequences coding the capsid domain and the amino terminus of the matrix domain of Gag respectively (FIG. 2A-2B). Both hybrids were designed to have 8-nt ssRNA toeholds. Transfection of the individual hybrids showed no significant effect on relative virus release. However, the co-transfection of cognate hybrids reduced the production of virus significantly (FIG. 2C).

In certain embodiments, the design rationale of R/DNA hybrids is the following: functional Dicer substrate siRNAs are split between ssRNA toeholds of two cognate R/DNA hybrids preventing them from being diced and thus, making them non-functional.

#### **ssRNA Toehold Interaction**

The RNA-based nanoparticles, e.g., RNA/DNA hybrid particles, comprise in certain preferred embodiments single strand RNA toehold sequences, particularly to enhance the interaction and reassociation of RNA/RNA strand pairs as between an RNA/DNA hybrid particle and a cognate RNA/DNA hybrid molecule.

Without being bound by theory, hybridization of the invading strand is initiated at a short single stranded "toehold" domain attached to one end of the substrate, leading to a branch migration reaction that displaces the target strand from the substrate.

The term "single strand RNA toehold" refers to nucleation site of a domain comprising an RNA sequence designed to initiate hybridization of the domain with a complementary RNA sequence. The secondary structure of a nanoparticle may be such that the toehold is exposed or sequestered. For example, in some embodiments, the secondary structure of the toehold is such that the toehold is available to hybridize to a complementary nucleic acid (the toehold is "exposed," or "accessible"), and in other embodiments, the secondary structure of

the toehold is such that the toehold is not available to hybridize to a complementary nucleic acid (the toehold is "sequestered," or "inaccessible"). If the toehold is sequestered or otherwise unavailable, the toehold can be made available by some event such as, for example, the opening of the hairpin of which it is a part of. When exposed, a toehold is configured such that a complementary nucleic acid sequence can nucleate at the toehold.

A scheme of re-association for the hybrids is described in PCT/US2012/065945, which is incorporated by reference in its entirety herein. The complementary single-stranded unzipped toeholds in R/DNA hybrids are designed using Mfold (Zuker, M, *Nucleic Acids Res* 31, 3406-3415 (2003)) to avoid any stable secondary structures. In order to exceed a melting temperature ( $T_m$ ) of 37°C, the minimal length of the unzipped toeholds with GC content >60 should be at least 12 nucleotides (nts). The  $T_m$  for designed single stranded toeholds is estimated to be ~ 40 °C using the Wallace rule (Wallace, R.B. et al., *Nucleic Acids Res* 6, 3543-3557 (1979)).

### **Computational Prediction**

In certain embodiments, an advanced algorithm has been used for the computational predictions of R/DNA hybrid re-associations and RNA secondary structures. In-silico secondary structure predictions are required for the further advancement of RNA based nanoparticles by understanding and utilizing the interactions of RNA strands with non-nested base pairing. The novel computational approach (which can be referred to herein in one embodiment as "HyperFold") is capable of predicting base pairing of RNA/RNA, DNA/DNA and RNA/DNA hybrid interactions.

A variety of secondary structure prediction programs for multiple RNA strands have been already reported in literature (Lorenz et al, *Algorithms Mol Biol* 2011, 6, 26; Cao et al, *RNA* 2014, 20, 835; Bernhart et al, *Algorithms Mol Biol* 2006, 1, 3; Bindewald et al, *ACS Nano* 2011, 5, 9542; Andronescu et al, *J Mol Biol* 2005, 345, 987). The NUPACK software is

in addition to RNA/RNA interactions also capable of predicting DNA/DNA interactions (Dirks et al, *J Comput Chem* 2003, 24, 1664; Zadeh et al, *J Comput Chem* 2010, 32, 170). Secondary structure predictions of RNA/DNA hybrid duplexes have been made available via the RNA Vienna package (Lorenz et al, *Algorithms Mol Biol* 2011, 6, 26).

The software described here is unique in the sense that it allows for secondary structure predictions of multiple nucleic acids strands simultaneously taking into account possible RNA/RNA, RNA/DNA and DNA/DNA interactions which may include pseudoknots. RNA structures with non-nested base pairings (in other words secondary structures whose circular diagram representations contain “crossing arcs”) are referred to as pseudoknotted (Bindewald et al, *ACS Nano* 2011, 5, 9542). The presented prediction approach does consider base pairs that are non-nested with respect to each other even if the involved nucleotides involve more than one nucleotide strand.

HyperFold performs competitively compared to other published folding algorithms. A large variety of RNA/DNA hybrid re-association experiments were performed for different concentrations, RNA toehold lengths, and G+C content and it was observed that tendencies for re-association correspond well to computational predictions.

The computational structure prediction approach was also used to predict equilibrium concentrations of complexes consisting of RNA/DNA hybrid sequences. For all toehold lengths and concentrations, it was found computationally that the re-associated RNA and DNA duplexes correspond to the lowest free energy conformation. In other words, the computational results suggest, that experimentally observed dramatic differences in re-association are due to kinetic effects. This is also confirmed by experimental results where re-association is observed for RNA/DNA hybrids without toeholds provided they have high concentration. The computationally estimated values when plotted together with the experimentally determined re-association rates then it corresponds to different concentrations

and toehold lengths, follows fairly well a sigmoidal curve as one would expect for a chemical system that contains a kinetic barrier.

### **Conjugation to Nanoparticles**

The nanoparticles described herein may also be configured for delivering agents. For example, nanoparticles can be used to deliver one or more agents that are selected from one or more of the group consisting of: siRNAs, RNA or DNA aptamers, fluorescent dyes, small molecules, RNA-DNA hybrids with split functionalities, split lipase, split GFP, proteins, therapeutic agents and imaging agents.

The compositions of the present invention have therapeutic uses. Any number of diseases or disorders can be treated by the compositions of the present invention and may be limited, in fact, only by the agent or agents that can be loaded in the inside of the nanoparticle or conjugated to the outside.

For example, RNA NPs can be engineered to carry multiple siRNAs against different disease targets. In one exemplary embodiment, six different siRNAs against different parts of the HIV-1 genome can be used for combinatorial RNAi therapy. The invention is not limited HIV, or to any disease or group of diseases, but is rather defined by the siRNAs that can be used to treat particular diseases. This concept of targeting a specific pathway upon the presence of a particular RNA in the cytoplasm can be applied to cancer (including cancer stem cells) or RNA viruses in general (e.g. Flaviviruses, Alphaviruses). HAART therapy as it currently exists, can successfully suppress virus replication within the human host. With this approach, however, it is currently not possible to eradicate the HIV virus from an infected patient because approved HIV drugs act as virus suppressors and do not kill human cells that are infected by the virus. The present invention can also lead to a novel anti-viral drug that has the unique feature of selectively killing HIV infected cells using appropriate aptamers, for cell targeting, that are associated with RNA NPs containing specific siRNAs or RNA/DNA



siRNA hybrids. The guide strands are designed to be an antisense to human apoptosis inhibitor genes (BCL-2, FLIP, STAT3, XIAP, SURVIVIN, etc). Thus, the activation of RNAi (RNA interference pathway) will result in apoptosis of the HIV-infected cell. In addition, in a more general sense, the siRNA targets may include cancer related genes, for example, but not limited to, the hypoxia pathway: Hif1alpha, VEGF; DNA repair pathway: PARP; microRNAs: miR21, miR7, miR128a, miR210; cancer stem cells: genes in NOTCH, HEDGEHOG, PTEN, WNT, TGFbeta pathways; immune modulation: Interleukin (IL-6, IL-10) and genes in the JAK/STAT, SMAD, TNFalpha. In principle the concept can be expanded to include any genetically related diseases.

Exemplary potential applications of multi-functional nanoparticles of the invention in which 2, 3, 4, or more agents are coupled to a nanoparticle include using one or more agents to target a macromolecular structure or a cell and using the second one to alter the function/properties of the macromolecule or cell, e.g., using a protein to target a cell and using a toxin or cell death protein to kill the targeted cell, using an siRNA to silence genes, or using a fluorescent particle for visualization, or using a chemical or protein to target a protein within a complex and another one to alter the function of a different component of the complex.

In certain embodiments, the nanoparticle comprises one or more agents. In further preferred embodiments, the agent can be conjugated to the nanoparticle. Conjugated can be understood as attached, linked, mixed, or otherwise present on or in a magnetoliposome. For example, an agent can be conjugated by covalent or ionic linkage, by use of a chelate or other linker moiety. As used herein, conjugation of an agent to a nanoparticle does not disrupt the desired activity of the agent.

The agent can comprise any material or compound or composition or agent for in vivo or in vitro use for imaging, diagnostic or therapeutic treatment that can be enclosed in the inside the nanoparticle or can be conjugated with the nanoparticle without appreciably

disturbing the physical integrity of the nanoparticle. A nanoparticle can comprise one or more agents of one or more types. For example, a nanoparticle can comprise a therapeutic agent, and the targeting of the agent can be followed by further conjugation with an imaging agent. Similarly, cocktails of therapeutic agents are typically used in the treatment of cancer. A nanoparticle can comprise more than one type of therapeutic agent.

Examples of agents include inhibitory nucleic acids, including but not limited to siRNAs, RNA or DNA aptamers, fluorescent dyes, small molecules, RNA-DNA hybrids with split functionalities, split lipase, split GFP, proteins, therapeutic agents and imaging agents (for example gadolinium, manganese, chromium, or iron).

In certain embodiments, the NP molecules described herein operate by forming inhibitory nucleic acid molecules once in target cells. Such inhibitory nucleic acids include single and double stranded nucleic acid molecules (e.g., DNA, RNA, and analogs thereof) that bind a nucleic acid molecule that encodes target RNA (e.g., antisense oligonucleotide molecules, siRNA, shRNA) as well as nucleic acid molecules that bind directly to a target polypeptide to modulate its biological activity (e.g., aptamers).

Catalytic RNA molecules or ribozymes that include an antisense target RNA sequence of the present disclosure can be used to inhibit expression of target RNAs in vivo. The inclusion of ribozyme sequences within antisense RNAs confers RNA-cleaving activity upon them, thereby increasing the activity of the constructs. The design and use of target RNA-specific ribozymes is described in Haseloff et al., *Nature* 334:585-591. 1988, and U.S. Patent Application Publication No. 2003/0003469 A1, each of which is incorporated by reference

The disclosure also features a catalytic RNA molecule that includes, in the binding arm, an antisense RNA having between eight and nineteen consecutive nucleobases. In preferred embodiments of this disclosure, the catalytic nucleic acid molecule is formed in a hammerhead or hairpin motif. Examples of such hammerhead motifs are described by Rossi

et al., *Aids Research and Human Retroviruses*, 8:183, 1992. Example of hairpin motifs are described by Hampel et al., "RNA Catalyst for Cleaving Specific RNA Sequences," filed Sep. 20, 1989, which is a continuation-in-part of U.S. Ser. No. 07/247,100 filed Sep. 20, 1988, Hampel and Tritz, *Biochemistry*, 28:4929, 1989, and Hampel et al., *Nucleic Acids Research*, 18: 299, 1990. These specific motifs are not limiting in the disclosure and those skilled in the art will recognize that all that is important in an enzymatic nucleic acid molecule of this disclosure is that it has a specific substrate binding site which is complementary to one or more of the target gene RNA regions, and that it have nucleotide sequences within or surrounding that substrate binding site which impart an RNA cleaving activity to the molecule.

Small hairpin RNAs consist of a stem-loop structure with optional 3' UU-overhangs. While there may be variation, stems can range from 21 to 31 bp (desirably 25 to 29 bp), and the loops can range from 4 to 30 bp (desirably 4 to 23 bp). For expression of shRNAs within cells, plasmid vectors containing either the polymerase III H1-RNA or U6 promoter, a cloning site for the stem-looped RNA insert, and a 4-5-thymidine transcription termination signal can be employed. The Polymerase III promoters generally have well-defined initiation and stop sites and their transcripts lack poly(A) tails. The termination signal for these promoters is defined by the polythymidine tract, and the transcript is typically cleaved after the second uridine. Cleavage at this position generates a 3' UU overhang in the expressed shRNA, which is similar to the 3' overhangs of synthetic siRNAs. Additional methods for expressing the shRNA in mammalian cells are described in the references cited above.

#### siRNA

By "siRNA" is meant a double stranded RNA. Optimally, an siRNA is 18, 19, 20, 21, 22, 23, 24 or more nucleotides in length and has a 2 base overhang at its 3' end. It is understood that the term "siRNA" includes both diceable and non-diceable siRNAs. These

dsRNAs can be introduced to an individual cell or to a whole animal; for example, they may be introduced systemically via the bloodstream. Such siRNAs are used to downregulate mRNA levels or promoter activity. Functional siRNAs can be released by Dicer nuclease. Short twenty-one to twenty-five nucleotide double-stranded RNAs are effective at down-regulating gene expression (Zamore et al., Cell 101: 25-33; Elbashir et al., Nature 411: 494-498, 2001, hereby incorporated by reference). The therapeutic effectiveness of an siRNA approach in mammals was demonstrated in vivo by McCaffrey et al. (Nature 418: 38-39, 2002).

Given the sequence of a target gene, siRNAs may be designed to inactivate that gene. Such siRNAs, for example, could be administered directly to an affected tissue, or administered systemically. The nucleic acid sequence of an *Parl* gene can be used to design small interfering RNAs (siRNAs). The 21 to 25 nucleotide siRNAs may be used, for example, as therapeutics to inhibit disease related genes.

The inhibitory nucleic acid molecules of the present disclosure may be employed as double-stranded RNAs for RNA interference (RNAi)-mediated knock-down of target RNA expression. In therapeutic embodiments, the target RNA is a disease related gene. For example, in a non-limiting embodiment, the target RNA is a gene that is involved in HIV. In another embodiment, the target RNA gene is a gene that is involved in cancer development or progression. In another embodiment, target RNA expression is reduced in a virus infected cell. In another embodiment, the target RNA encodes apoptosis inhibitor proteins and the cells are infected with HIV. RNAi is a method for decreasing the cellular expression of specific proteins of interest (reviewed in Tuschl, ChemBioChem 2:239-245, 2001; Sharp, Gene Dev 15:485-490, 2000; Hutvagner and Zamore, Curr Opin Genet Devel 12:225-232, 2002; and Hannon, Nature 418:244-251, 2002). The introduction of siRNAs into cells either

by transfection of dsRNAs or through expression of siRNAs using a plasmid-based expression system is increasingly being used to create loss-of-function phenotypes in mammalian cells.

In one embodiment of the disclosure, a double-stranded RNA (dsRNA) molecule is made that includes between eight and nineteen consecutive nucleobases of a nucleobase oligomer of the disclosure. The dsRNA can be two distinct strands of RNA that have duplexed, or a single RNA strand that has self-duplexed (small hairpin (sh)RNA). Typically, dsRNAs are about 21 or 22 base pairs, but may be shorter or longer (up to about 29 nucleobases) if desired. dsRNA can be made using standard techniques (e.g., chemical synthesis or in vitro transcription). Kits are available, for example, from Ambion (Austin, Tex.) and Epicentre (Madison, Wis.). Methods for expressing dsRNA in mammalian cells are described in Brummelkamp et al. *Science* 296:550-553, 2002; Paddison et al. *Gene Dev* 16:948-958, 2002. Paul et al. *Nat Biotechnol* 20:505-508, 2002; Sui et al. *Proc Natl Acad Sci USA* 99:5515-5520, 2002; Yu et al. *Proc Natl Acad Sci USA* 99:6047-6052, 2002; Miyagishi et al. *Nat Biotechnol* 20:497-500, 2002; and Lee et al. *Nat Biotechnol* 20:500-505, 2002, each of which is hereby incorporated by reference. In certain embodiments, the sense strand of the double stranded siRNA is split into two smaller oligonucleotides, also referred to as three stranded siRNA.

Small hairpin RNAs consist of a stem-loop structure with optional 3' UU-overhangs. While there may be variation, stems can range from 21 to 31 bp (desirably 25 to 29 bp), and the loops can range from 4 to 30 bp (desirably 4 to 23 bp). For expression of shRNAs within cells, plasmid vectors containing either the polymerase III H1-RNA or U6 promoter, a cloning site for the stem-looped RNA insert, and a 4-5-thymidine transcription termination signal can be employed. The Polymerase III promoters generally have well-defined initiation and stop sites and their transcripts lack poly(A) tails. The termination signal for these promoters is defined by the polythymidine tract, and the transcript is typically cleaved after the second

uridine. Cleavage at this position generates a 3' UU overhang in the expressed shRNA, which is similar to the 3' overhangs of synthetic siRNAs. Additional methods for expressing the shRNA in mammalian cells are described in the references cited above.

The invention encompasses stabilized R/DNA NPs having modifications that protect against 3' and 5' exonucleases as well as endonucleases. Such modifications desirably maintain target affinity while increasing stability in vivo. In various embodiments, R/DNA NPs of the invention include chemical substitutions at the ribose and/or phosphate and/or base positions of a given nucleobase sequence. For example, R/DNA NPs of the invention include chemical modifications at the 2' position of the ribose moiety, circularization of the aptamer, 3' capping and 'spiegelmer' technology. R/DNA NPs having A and G nucleotides sequentially replaced with their 2'-OCH<sub>3</sub> modified counterparts are particularly useful in the methods of the invention. Such modifications are typically well tolerated in terms of retaining affinity and specificity. In various embodiments, R/DNA NPs include at least 10%, 25%, 50%, or 75% modified nucleotides. In other embodiments, as many as 80-90% of the R/DNA NPs' nucleotides contain stabilizing substitutions. In other embodiments, 2'-OMe containing R/DNA NPs are synthesized. Such R/DNA NPs are desirable because they are inexpensive to synthesize and natural polymerases do not accept 2'-OMe nucleotide triphosphates as substrates so that 2'-OMe nucleotides cannot be recycled into host DNA. Using methods described herein, R/DNA NPs will be selected for increased in vivo stability. In one embodiment, R/DNA NPs having 2'-F and 2'-OCH<sub>3</sub> modifications are used to generate nuclease resistant aptamers. In other embodiments, the nucleic acids of the invention have one or more locked nucleic acids (LNA). LNA refers to a modified RNA nucleotide. The ribose of the LNA is modified with an extra bridge connecting the 2' oxygen and the 4' carbon which locks the ribose into the North or 3'-endo conformation. See e.g., Kaur, H. et al., *Biochemistry*, vol. 45, pages 7347-55; and Koshkin, A.A., et al., *Tetrahedron*, vol. 54,

pages 3607-3630. In other embodiments, one or more nucleic acids of the invention incorporate a morpolino structure where the nucleic acid bases are bound to morpholine rings instead of deoxyribose rings and are linked through phosphorodiamidate groups instead of phosphates. See e.g., Summerton, J. and Weller, D., *Antisense & Nucleic Acid Drug Development*, vol. 7, pages 187-195. Yet other modifications, include (PS)-phosphate sulfur modifications wherein the phosphate backbone of the nucleic acid is modified by the substitution of one or more sulfur groups for oxygen groups in the phosphate backbone. Other modifications that stabilize nucleic acids are known in the art and are described, for example, in U.S. Patent Nos. 5,580,737; and in U.S. Patent Application Publication Nos. 20050037394, 20040253679, 20040197804, and 20040180360.

The agent may be a RNA or DNA aptamer. An aptamer is a stable DNA, RNA, or peptide that binds with high affinity and specificity to targets such as small organics, peptides, proteins, cells, and tissues. Unlike antibodies, some aptamers exhibit stereoselectivity. The present invention is not limited to any particular aptamer, but rather can be any aptamer known in the art to be useful in treating a disease or condition. For example, the Aptamer Database is a comprehensive, annotated repository for information about aptamers and in vitro selection. This resource is provided to collect, organize and distribute all the known information regarding aptamer selection, and is publicly available at <http://aptamer.icmb.utexas.edu/>.

The agent may be RNA-DNA hybrids with split functionalities, as described *infra*.

The agent may also be a targeting agent that directs the nanoparticle to a delivery site. For example, the targeting agent may be a ligand, e.g. a peptide ligand that has specific cell surface binding partners, e.g., ligand receptors, that are preferentially exhibited on the surface of a target cell. As used herein, "receptor" and "ligand" refer to two members of a specific binding pair that are binding partners. A receptor is that member of the pair that is found



localized on the surface of the target; the ligand is the member of the pair that is found on the surface of the nanoparticle. Accordingly, in certain embodiments, the invention features a nanoparticle comprising a member of a binding pair, or a fragment thereof that retains the capacity to specifically bind the other member of the binding pair, on its surface and the other member of that binding pair, or a fragment thereof that retains the capacity to specifically bind its partner, is present on the surface of a target. In certain embodiments, the targeting agent may be an antibody, for example a single-chain antibody, for which a binding partner would include an antigen thereof, or a fragment, derivative or variant thereof that retains the capacity to bind to the single-chain antibody.

A therapeutic agent may be a molecule, atom, ion, receptor and/or other entity which is capable of detecting, identifying, inhibiting, treating, catalyzing, controlling, killing, enhancing or modifying a target such as a protein, glyco protein, lipoprotein, lipid, a targeted cell, a targeted organ, or a targeted tissue.

In certain cases, the therapeutic agent is a radiotherapeutic agent, and can be selected from, but is not limited to radioactive gadolinium, radioactive boron, and radioactive iodine.

In certain examples, the agent can be, but is not limited to: drugs, such as antibiotics, analgesics, hypertensives, cardiotonics, and the like, such as acetaminaphen, acyclovir, alkeran, amikacin, ampicillin, aspirin, bisantrene, bleomycin, neocardiostatin, carboplatin, chloroambucil, chloramphenicol, cytarabine, daunomycin, doxorubicin, fluorouracil, gentamycin, ibuprofen, kanamycin, meprobamate, methotrexate, novantrone, nystatin, oncovin, phenobarbital, polymyxin, probucol, procarbazine, rifampin, streptomycin, spectinomycin, symmetrel, thioguanine, tobramycin, temozolamide, trimethoprim, cisplatin, oxaliplatin, mechlorethamine, cyclophosphamide, chlorambucil, azathioprine, mercaptopurine, vinca alkaloids, taxanes, vincristine, vinblastine, vinorelbine, vindesine, etoposide, teniposide, paclitaxel, irinotecan, topotecan, amsacrine, etoposide, etoposide

phosphate, teniposide, and dactinomycin and valban; diphtheria toxin, gelonin, exotoxin A, abrin, modeccin, ricin, radioactive gadolinium, radioactive boron, and radioactive iodine; or toxic fragments thereof; metal ions, such as the alkali and alkaline-earth metals; radionuclides, such as those generated from actinides or lanthanides or other similar transition elements or from other elements, such as  $^{51}\text{Cr}$ ,  $^{47}\text{Sc}$ ,  $^{67}\text{Cu}$ ,  $^{67}\text{Ga}$ ,  $^{82}\text{Rb}$ ,  $^{89}\text{Sr}$ ,  $^{88}\text{Y}$ ,  $^{90}\text{Y}$ ,  $^{99\text{m}}\text{Tc}$ ,  $^{105}\text{Rh}$ ,  $^{109}\text{Pd}$ ,  $^{111}\text{In}$ ,  $^{115\text{m}}\text{In}$ ,  $^{125}\text{I}$ ,  $^{131}\text{I}$ ,  $^{140}\text{Ba}$ ,  $^{140}\text{La}$ ,  $^{149}\text{Pm}$ ,  $^{153}\text{Sm}$ ,  $^{159}\text{Gd}$ ,  $^{166}\text{Ho}$ ,  $^{175}\text{Yb}$ ,  $^{177}\text{Lu}$ ,  $^{186}\text{Re}$ ,  $^{188}\text{Re}$ ,  $^{194}\text{Ir}$ , and  $^{199}\text{Au}$ ; signal generators, which includes anything that results in a detectable and measurable perturbation of the system due to its presence, such as fluorescing entities, phosphorescence entities and radiation; signal reflectors, such as paramagnetic entities, for example, Fe, Gd, Cr, or Mn; chelated metal, such as any of the metals given above, whether or not they are radioactive, when associated with a chelant; signal absorbers, such as contrast agents and electron beam opacifiers, for example, Fe, Gd, Cr, or Mn; antibodies, including monoclonal antibodies and anti-idiotypic antibodies; antibody fragments; hormones; biological response modifiers such as interleukins, interferons, viruses and viral fragments; diagnostic opacifiers; and fluorescent moieties. Other pharmaceutical materials include scavenging agents such as chelants, antigens, antibodies or any moieties capable of selectively scavenging therapeutic or diagnostic agents.

Other examples of therapeutic agents include antimicrobial agents, analgesics, antiinflammatory agents, counterirritants, coagulation modifying agents, diuretics, sympathomimetics, anorexics, antacids and other gastrointestinal agents; antiparasitics, antidepressants, antihypertensives, anticholinergics, stimulants, antihormones, central and respiratory stimulants, drug antagonists, lipid-regulating agents, uricosurics, cardiac glycosides, electrolytes, ergot and derivatives thereof, expectorants, hypnotics and sedatives, antidiabetic agents, dopaminergic agents, antiemetics, muscle relaxants, parasympathomimetics, anticonvulsants, antihistamines, beta-blockers, purgatives,

antiarrhythmics, contrast materials, radiopharmaceuticals, antiallergic agents, tranquilizers, vasodilators, antiviral agents, and antineoplastic or cytostatic agents or other agents with anticancer properties, or a combination thereof. Other suitable therapeutic moieties include contraceptives and vitamins as well as micro- and macronutrients. Still other examples include antiinfectives such as antibiotics and antiviral agents; analgesics and analgesic combinations; anorexics; antiheimintics; antiarthritics; antiasthmatic agents; anticonvulsants; antidepressants; antidiuretic agents; antidiarrheals; antihistamines; antiinflammatory agents; antimigraine preparations; antinauseants; antineoplastics; antiparkinsonism drugs; antipruritics; antipsychotics; antipyretics, antispasmodics; anticholinergics; sympathomimetics; xanthine derivatives; cardiovascular preparations including calcium channel blockers and beta-blockers such as pindolol and antiarrhythmics; antihypertensives; diuretics; vasodilators including general coronary, peripheral and cerebral; central nervous system stimulants; cough and cold preparations, including decongestants; hormones such as estradiol and other steroids, including corticosteroids; hypnotics; immunosuppressives; muscle relaxants; parasympatholytics; psychostimulants; sedatives; and tranquilizers; and naturally derived or genetically engineered proteins, polysaccharides, glycoproteins, or lipoproteins.

Nanoparticles may be directed to target sites. Preferred target sites comprise cancer cells, solid tumors, sites of inflammation and damaged bone or tissue.

For example, nanoparticle may further comprise an antibody or a peptide that acts as a targeting moiety to enable specific binding to a target cell bearing a target molecule, e.g., a cell surface marker to which the antibody or peptide is directed or a disease-specific marker to which the antibody or peptide is directed. The nanoparticle may further comprise a nucleotide, e.g. an oligonucleotide that acts as a targeting moiety to enable specific binding to

a target cell bearing a target molecule. For example, the oligonucleotide may be an aptamer that binds a specific target molecule.

Further exemplary potential applications of the multi-functional nanoparticles of the invention include use of the nanoparticles as riboswitch aptamers, ribozymes, or beacons.

Riboswitches are a type of control element that use untranslated sequence in an mRNA to form a binding pocket for a metabolite that regulates expression of that gene. Riboswitches are dual function molecules that undergo conformational changes and that communicate metabolite binding typically as either increased transcription termination or reduced translation efficiency via an expression platform.

Ribozymes catalyze fundamental biological processes, such as RNA cleavage by transesterification. The polyvalent RNA nanoparticles of the invention can be incorporated in to ribozymes using methods described in, for example, US Patent No. 6,916,653, incorporated by reference in its entirety herein.

A number of "molecular beacons" (often fluorescence compounds) can be attached to RNA nanoparticles of the invention to provide a means for signaling the presence of, and quantifying, a target analyte. Molecular beacons, for example, employ fluorescence resonance energy transfer-based methods to provide fluorescence signals in the presence of a particular analyte/biomarker of interest. In preferred embodiments, the term "molecular beacon" refers to a molecule or group of molecules (i.e., a nucleic acid molecule hybridized to an energy transfer complex or chromophore(s)) that can become detectable and can be attached to a nanoparticle under preselected conditions. Similarly, amplifying fluorescent polymers (AFPs) can be utilized in the present invention. An AFP is a polymer containing several chromophores that are linked together. As opposed to isolated chromophores that require 1:1 interaction with an analyte in conventional fluorescence detection, the fluorescence of many chromophores in an AFP can be influenced by a single molecule. For

example, a single binding event to an AFP can quench the fluorescence of many polymer repeat units, resulting in an amplification of the quenching. Quenching is a process which decreases the intensity of the fluorescence emission. Molecular beacons and AFPs, including their methods for preparation, that can be used in the present invention are described in numerous patents and publications, including U.S. Pat. No. 6,261,783.

Any protein can be coupled to nanoparticles. For instance, glycoproteins are most easily coupled, as they can be oxidized to generate an active aldehyde group. Other proteins can be coupled via their --COOH group(s) but with lower efficiency. However, other means known in the art, such as di-imide reagents, e.g. carbodiimide can be used to couple proteins lacking sugars to the nanoparticles.

Polyethylene Glycol (PEG) chains can be conjugated to the nanoparticles. PEG chains render the nanotubes highly water-soluble. PEG-phospholipids (PEG-PL) have been used in the formation of micelles and liposomes for drug delivery (Adlakha-Hutcheon, G.; Bally, M. B.; Shew, C. R.; Madden, T. D. *Nature Biotech.* 1999, 17, 775-779; Meyer, O.; Kirpotin, D.; Hong, K.; Sternberg, B.; Park, J. W.; Woodle, M. C.; Papahadjopoulos, D. J. *Biol. Chem.* 1998, 273, 15621-15627; Papahadjopoulos, D.; Allen, T. M.; Gabizon, A.; Mayhew, E.; Matthay, K.; Huang, S. K.; Lee, K. D.; Woodle, M. C.; Lasic, D. D.; Redemann, C.; Martin, F. J. *Proc. Nat. Acad. Sci. USA.* 1991, 88, 11460-11464).

Functional groups can be coupled to the nanoparticle, for instance the functional group can be a reactive functional group. Suitable functional groups include, but are not limited to, a haloacetyl group, an amine, a thiol, a phosphate, a carboxylate, a hydrazine, a hydrazide an aldehyde or a combination thereof. Other functional groups include groups such as a reactive functionality or a complementary group. In addition, RNA functional groups can be attached, as for example ribozymes or riboswitch aptamers.

The nanoparticle can be used for attachment of small molecules for specific interactions with nucleic acids, carbohydrates, lipids, proteins, antibodies, or other ligands.

The nanoparticle can have dyes attached. The dye is can be a fluorescent dye, or a plurality of fluorescent dyes. Suitable dyes include, but are not limited to, YOYO-1, JOJO-1, LOLO-1, YOYO-3, TOTO, BOBO-3, SYBR, SYTO, SYTOX, PicoGreen, OliGreen, and combinations thereof. Other dyes include, thiazole orange, oxazole yellow, or non-intercalating dyes such as fluorescein, rhodamine, cyanine or coumarin based dyes, and combinations thereof. Other suitable dyes include, but are not limited to, 4-acetamido-4'-isothiocyanatostilbene-2,2'-disulfonic acid; acridine and derivatives: acridine, acridine isothiocyanate; 5-(2'-aminoethyl)aminonaphthalene-1-sulfonic acid (EDANS); 4-amino-N-[3-vinylsulfonyl]phenyl]naphthalimide-3,5 disulfonate; N-(4-anilino-1-naphthyl)maleimide; anthranilamide; BODIPY; Brilliant Yellow; coumarin and derivatives: coumarin, 7-amino-4-methylcoumarin (AMC, Coumarin 120), 7-amino-4-trifluoromethylcoumarin (Coumarin 151); cyanine dyes; cyanosine; 4',6-diaminidino-2-phenylindole (DAPI); 5',5"-dibromopyrogallol-sulfonaphthalein (Bromopyrogallol Red); 7-diethylamino-3-(4'-isothiocyanatophenyl)-4-methylcoumarin; diethylenetriamine pentaacetate; 4,4'-diisothiocyanatodihydro-stilbene-2,2'-disulfonic acid; 4,4'-diisothiocyanatostilbene-2,2'-disulfonic acid; 5-[dimethylamino]naphthalene-1-sulfonyl chloride (DNS, dansylchloride); 4-dimethylaminophenylazophenyl-4'-isothiocyanate (DABITC); eosin and derivatives: eosin, eosin isothiocyanate, erythrosin and derivatives: erythrosin B, erythrosin, isothiocyanate; ethidium; fluorescein and derivatives: 5-carboxyfluorescein (FAM), 5-(4,6-dichlorotriazin-2-yl)aminofluorescein (DTAF), 2',7'-dimethoxy-4',5'-dichloro-6-carboxyfluorescein (JOE), fluorescein, fluorescein isothiocyanate, QFITC, (XRITC); fluorescamine; IR144; IR1446; Malachite Green isothiocyanate; 4-methylumbelliferoneortho cresolphthalein; nitrotyrosine; pararosaniline; Phenol Red; B-phycoerythrin; o-phthalaldehyde; pyrene and derivatives:

pyrene, pyrene butyrate, succinimidyl 1-pyrene; butyrate quantum dots; Reactive Red 4 (Cibacron.TM. Brilliant Red 3B-A) rhodamine and derivatives: 6-carboxy-X-rhodamine (ROX), 6-carboxyrhodamine (R6G), lissamine rhodamine B sulfonyl chloride rhodamine (Rhod), rhodamine B, rhodamine 123, rhodamine X isothiocyanate, sulforhodamine B, sulforhodamine 101, sulfonyl chloride derivative of sulforhodamine 101 (Texas Red); N,N,N',N'-tetramethyl-6-carboxyrhodamine (TAMRA); tetramethyl rhodamine; tetramethyl rhodamine isothiocyanate (TRITC); riboflavin; rosolic acid; terbium chelate derivatives; Cy 3; Cy 5; Cy 5.5; Cy 7; IRD 700; IRD 800; La Jolla Blue; phthalocyanine; and naphthalo cyanine. Suitable dyes for use in the nanoparticles of the present invention include, without limitation, a family of homodimeric cyanine DNA intercalating dyes from Molecular Probes that cover the visible spectrum, such as YOYO-1 (488/509), JOJO-1 (532/545), LOLO-1 (565/579), and YOYO-3 (612/631), SYBR-101 (488/505) and SYTO-62 (652/676). Given sufficient detection SN, dyes are mixed in various ratios in a single particle such that, for example, different fluorescence spectra are obtained from mixtures of just 2 dyes.

According to the invention, one or more therapeutic, diagnostic, or delivery agents are directly included in the building block sequences. In certain embodiments, the delivery agent can be a targeting agent. Targeting agents are used to direct the nanoparticle to a tissue or cell target. An exemplary embodiment of a targeting agent is an antibody. For example, antibodies suitable for use as targeting agents in the present invention include antibodies directed to cell surface antigens which cause the antibody-nanoparticle complex to be internalized, either directly or indirectly. For example, in the treatment of cancer, suitable antibodies include antibodies to CD33 and CD22. CD33 and CD22 that are over-expressed and dimerized on lymphomas.

In certain preferred embodiments of the invention biotin is conjugated to the nanoparticle. For example, the nanoparticles of the invention can be further functionalized



using biotin-streptavidin interactions to immobilize molecules inside or outside the polyhedra, e.g. polyhedral cages. For example, streptavidin can be conjugated to guanosine mono-phosphothioate (GMPS)-modified tectoRNAs by means of a biotin linker. In certain preferred embodiments, the biotin linker is incorporated to a mono-phosphothioate at the 5' position of tectoRNAs.

A wide variety of particle sizes are suitable for the present invention. In certain aspects, the particle has a diameter of about 10 nanometers to about 10 microns. Preferably the particle diameter is about 10 to 700 nanometers, and more preferably, the diameter of about 10 nanometers to about 100 nanometers.

The polyvalent RNA nanoparticle or the polyvalent RNA nanotube as described herein has a number of uses. For example, the polyvalent RNA nanoparticle or the polyvalent RNA nanotube can be used in drug delivery, imaging, nanocircuits, cell growth surfaces, medical implants, medical testing, or gene therapy.

In one particular embodiment, the polyvalent RNA nanoparticle or the polyvalent RNA polyhedra, e.g., cages, as described can be used in biological meshes. In one exemplary embodiment, the invention as described herein may find use as a biosensor in, for example, pathogen detection. In one particular embodiment, self-assembling nano-meshes are used to attach biosensors for pathogen detection or for x-ray crystallography by placing multiple copies of a protein or functional RNAs, for example, on the mesh. Biosensors for pathogen detection are advantageously employed in bioterrorism capacities.

In another exemplary embodiment, the polyvalent nanoparticles of the invention, as described herein, are employed as skeletons or scaffolds for tissue growth. These uses are exemplary, and not considered to be limiting.

### **Compositions**

The invention, in part, pertains to a drug delivery composition comprising the nanoparticles or the activatable nanoparticle systems as described herein. The drug delivery composition of the invention can gain entry into a cell or tissue.

Advantageously, the drug delivery composition of the invention provides for a more controlled delivery of an active agent, especially a therapeutic agent, to a site of action at an optimum rate and therapeutic dose. Thus, improvements in therapeutic index may be obtained by modulating the distribution of the active ingredient in the body. Association of the active ingredient with a delivery system enables, in particular, its specific delivery to the site of action or its controlled release after targeting the action site. By reducing the amount of active ingredient in the compartments in which its presence is not desired, it is possible to increase the efficacy of the active ingredient, to reduce its toxic side effects and even modify or restore its activity.

It is understood by one of skill in the art that changing the base composition of RNA changes the half-life of RNA and thus the release of RNA from the composition. For instance, the composition can be modified to consist of fast release, slow release or a staged release of polyvalent RNA nanoparticle.

In certain preferred embodiments, the drug delivery composition can comprise a second therapeutic agent. In some embodiments, the composition comprising nanoparticles and the second therapeutic agent are administered simultaneously, either in the same composition or in separate compositions. In some embodiments, the nanoparticle composition and the second therapeutic agent are administered sequentially, i.e., the nanoparticle composition is administered either prior to or after the administration of the second therapeutic agent. The term "sequential administration" as used herein means that the drug in the nanoparticle composition and the second agent are administered with a time separation of more than about 15 minutes, such as more than about any of 20, 30, 40, 50, 60 or more

minutes. Either the nanoparticle composition or the chemotherapeutic agent may be administered first. The nanoparticle composition and the chemotherapeutic agent are contained in separate compositions, which may be contained in the same or different packages. In some embodiments, the administration of the nanoparticle composition and the second therapeutic agent are concurrent, i.e., the administration period of the nanoparticle composition and that of the second therapeutic agent overlap with each other. In some embodiments, the administration of the nanoparticle composition and the second therapeutic agent are non-concurrent. For example, in some embodiments, the administration of the nanoparticle composition is terminated before the second therapeutic agent is administered. In some embodiments, the administration of the second therapeutic agent is terminated before the nanoparticle composition is administered. Administration may also be controlled by designing the RNA nanoparticle or nano-tube to have different half-lives. Thus, particle dissolution would be controlled by a timed release based upon variations in designed RNA stability.

The second therapeutic agent is selected from, but not limited to chemotherapeutic agents, cardiovascular drugs, respiratory drugs, sympathomimetic drugs, cholinomimetic drugs, adrenergic or adrenergic neuron blocking drugs, analgesics/antipyretics, anesthetics, antiasthmatics, antibiotics, antidepressants, antidiabetics, antifungals, antihypertensives, anti-inflammatories, antianxiety agents, immunosuppressive agents, immunomodulatory agents, antimigraine agents, sedatives/hypnotics, antianginal agents, antipsychotics, antimanic agents, antiarrhythmics, antiarthritic agents, antigout agents, anticoagulants, thrombolytic agents, antifibrinolytic agents, hemorheologic agents, antiplatelet agents, anticonvulsants, antiparkinson agents, antihistamines/antipruritics, agents useful for calcium regulation, antibacterials, antivirals, antimicrobials, anti-infectives, bronchodilators, hormones, hypoglycemic agents, hypolipidemic agents, proteins, peptides, nucleic acids, agents useful

for erythropoiesis stimulation, antiulcer/antireflux agents, antinauseants/antiemetics and oil-soluble vitamins, or combinations thereof.

When the second therapeutic agent is a chemotherapeutic agent, the chemotherapeutic agent is selected from, but not limited to, acivicin; aclarubicin; acodazole hydrochloride; acronine; adozelesin; aldesleukin; altretamine; ambomycin; ametantrone acetate; aminoglutethimide; amsacrine; anastrozole; anthramycin; asparaginase; asperlin; azacitidine; azetepa; azotomycin; batimastat; benzodepa; bicalutamide; bisantrene hydrochloride; bisnafide dimesylate; bizelesin; bleomycin sulfate; brequinar sodium; bropiramine; busulfan; cactinomycin; calusterone; caracemide; carbetimer; carboplatin; carmustine; carubicin hydrochloride; carzelesin; cedefingol; chlorambucil; cirolemycin; cisplatin; cladribine; crisnatol mesylate; cyclophosphamide; cytarabine; dacarbazine; dactinomycin; daunorubicin hydrochloride; decitabine; dexormaplatin; dezaguanine; dezaguanine mesylate; diaziquone; docetaxel; doxorubicin; doxorubicin hydrochloride; droloxifene; droloxifene citrate; dromostanolone propionate; duazomycin; edatrexate; eflornithine hydrochloride; elsamitrucin; enloplatin; enpromate; epipropidine; epirubicin hydrochloride; erbulozole; esorubicin hydrochloride; estramustine; estramustine phosphate sodium; etanidazole; etoposide; etoposide phosphate; etoprine; fadrozole hydrochloride; fazarabine; fenretinide; floxuridine; fludarabine phosphate; fluorouracil; flurocitabine; fosquidone; fostriecin sodium; gemcitabine; gemcitabine hydrochloride; hydroxyurea; idarubicin hydrochloride; ifosfamide; ilmofosine; interleukin II (including recombinant interleukin II, or rIL2), interferon alfa-2a; interferon alfa-2b; interferon alfa-n1; interferon alfa-n3; interferon beta-I a; interferon gamma-I b; iproplatin; irinotecan hydrochloride; lanreotide acetate; letrozole; leuprolide acetate; liarozole hydrochloride; lometrexol sodium; lomustine; losoxantrone hydrochloride; masoprocol; maytansine; mechlorethamine, mechlorethamine oxide hydrochloride rethamine hydrochloride; megestrol acetate; melengestrol acetate; melphalan; menogaril;

mercaptopurine; methotrexate; methotrexate sodium; metoprine; meturedopa; mitindomide; mitocarcin; mitocromin; mitogillin; mitomalcin; mitomycin; mitosper; mitotane; mitoxantrone hydrochloride; mycophenolic acid; nocodazole; nogalamycin; ormaplatin; oxisuran; paclitaxel; pegaspargase; peliomycin; pentamustine; peplomycin sulfate; perfosfamide; pipobroman; pipsulfan; piroxantrone hydrochloride; plicamycin; plomestane; porfimer sodium; porfiromycin; prednimustine; procarbazine hydrochloride; puromycin; puromycin hydrochloride; pyrazofurin; riboprine; rogletimide; safingol; safingol hydrochloride; semustine; simtrazene; sparfosate sodium; sparsomycin; spirogermanium hydrochloride; spiromustine; spiroplatin; streptonigrin; streptozocin; sulofenur; talisomycin; tecogalan sodium; tegafur; teloxantrone hydrochloride; temoporfin; teniposide; teroxirone; testolactone; thiamiprine; thioguanine; thiotepa; tiazofurin; tirapazamine; toremifene citrate; trestolone acetate; triciribine phosphate; trimetrexate; trimetrexate glucuronate; triptorelin; tubulozole hydrochloride; uracil mustard; uredopa; vapreotide; verteporfin; vinblastine sulfate; vincristine sulfate; vindesine; vindesine sulfate; vinepidine sulfate; vinglycinate sulfate; vinleurosine sulfate; vinorelbine tartrate; vinrosidine sulfate; vinzolidine sulfate; vorozole; zeniplatin; zinostatin; zorubicin hydrochloride, improsulfan, benzodepa, carboquone, triethylenemelamine, triethylenephosphoramidate, triethylenethiophosphoramidate, trimethylolomelamine, chlornaphazine, novembichin, phenesterine, trofosfamide, estermustine, chlorozotocin, gemzar, nimustine, ranimustine, dacarbazine, mannomustine, mitobronitol, aclacinomycins, actinomycin F(1), azaserine, bleomycin, carubicin, carzinophilin, chromomycin, daunorubicin, daunomycin, 6-diazo-5-oxo-1-norleucine, doxorubicin, olivomycin, plicamycin, porfiromycin, puromycin, tubercidin, zorubicin, denopterin, pteropterin, 6-mercaptopurine, ancitabine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, enocitabine, pulmozyme, aceglatone, aldophosphamide glycoside, bestrabucil, defofamide, demecolcine, elfornithine, elliptinium acetate, etoglucid, flutamide,

hydroxyurea, lentinan, phenamet, podophyllinic acid, 2-ethylhydrazide, razoxane, spirogermanium, tamoxifen, taxotere, tenuazonic acid, triaziquone, 2,2',2"-trichlorotriethylamine, urethan, vinblastine, vincristine, vindesine and related agents. 20-epi-1,25 dihydroxyvitamin D3; 5-ethynyluracil; abiraterone; aclarubicin; acylfulvene; adecypenol; adozelesin; aldesleukin; ALL-TK antagonists; altretamine; ambamustine; amidox; amifostine; aminolevulinic acid; amrubicin; amsacrine; anagrelide; anastrozole; andrographolide; angiogenesis inhibitors; antagonist D; antagonist G; antarelix; anti-dorsalizing morphogenetic protein-1; antiandrogen, prostatic carcinoma; antiestrogen; antineoplaston; antisense oligonucleotides; aphidicolin glycinate; apoptosis gene modulators; apoptosis regulators; apurinic acid; ara-CDP-DL-PTBA; arginine deaminase; asulacrine; atamestane; atrimustine; axinastatin 1; axinastatin 2; axinastatin 3; azasetron; azatoxin; azatyrosine; baccatin III derivatives; balanol; batimastat; BCR/ABL antagonists; benzochlorins; benzoylstauroporine; beta lactam derivatives; beta-alethine; betaclamycin B; betulinic acid; bFGF inhibitor; bicalutamide; bisantrene; bisaziridinylspermine; bisnafide; bistratene A; bizelesin; breflate; bropirimine; budotitane; buthionine sulfoximine; calcipotriol; calphostin C; camptothecin derivatives; canarypox IL-2; capecitabine; carboxamide-amino-triazole; carboxyamidotriazole; CaRest M3; CARN 700; cartilage derived inhibitor; carzelesin; casein kinase inhibitors (ICOS); castanospermine; cecropin B; cetorelix; chlorIns; chloroquinoxaline sulfonamide; cicaprost; cisporphyrin; cladribine; clomifene analogues; clotrimazole; collismycin A; collismycin B; combretastatin A4; combretastatin analogue; conagenin; crambescidin 816; crisnatol; cryptophycin 8; cryptophycin A derivatives; curacin A; cyclopentantraquinones; cycloplatam; cypemycin; cytarabine ocfosfate; cytolytic factor; cytostatin; daclicximab; decitabine; dehydrodidemnin B; deslorelin; dexamethasone; dexifosfamide; dexrazoxane; dexverapamil; diaziquone; didemnin B; didox; diethylnorspermine; dihydro-5-azacytidine; dihydrotaxol, 9-; dioxamycin; diphenyl

spiromustine; docetaxel; docosanol; dolasetron; doxifluridine; droloxifene; dronabinol; duocarmycin SA; ebselen; ecomustine; edelfosine; edrecolomab; eflornithine; elemene; emitefur; epirubicin; epristeride; estramustine analogue; estrogen agonists; estrogen antagonists; etanidazole; etoposide phosphate; exemestane; fadrozole; fazarabine; fenretinide; filgrastim; finasteride; flavopiridol; flezelastine; fluasterone; fludarabine; fluorodaunorubicin hydrochloride; forfenimex; formestane; fostriecin; fotemustine; gadolinium texaphyrin; gallium nitrate; galocitabine; ganirelix; gelatinase inhibitors; gemcitabine; glutathione inhibitors; hepsulfam; heregulin; hexamethylene bisacetamide; hypericin; ibandronic acid; idarubicin; idoxifene; idramantone; ilmofofosine; ilomastat; imidazoacridones; imiquimod; immunostimulant peptides; insulin-like growth factor-1 receptor inhibitor; interferon agonists; interferons; interleukins; iobenguane; iododoxorubicin; ipomeanol, 4-; iroplact; irsogladine; isobengazole; isohomohalicondrin B; itasetron; jasplakinolide; kahalalide F; lamellarin-N triacetate; lanreotide; leinamycin; lenograstim; lentinan sulfate; leptolstatin; letrozole; leukemia inhibiting factor; leukocyte alpha interferon; leuprolide+estrogen+progesterone; leuprorelin; levamisole; liarozole; linear polyamine analogue; lipophilic disaccharide peptide; lipophilic platinum compounds; lissoclinamide 7; lobaplatin; lombricine; lometrexol; lonidamine; losoxantrone; lovastatin; loxoribine; lurtotecan; lutetium texaphyrin; lysofylline; lytic peptides; maitansine; mannostatin A; marimastat; masoprocyl; maspin; matrilysin inhibitors; matrix metalloproteinase inhibitors; menogaril; merbarone; meterelin; methioninase; metoclopramide; MIF inhibitor; mifepristone; miltefosine; mirimostim; mismatched double stranded RNA; mitoguazone; mitolactol; mitomycin analogues; mitonafide; mitotoxin fibroblast growth factor-saporin; mitoxantrone; mofarotene; molgramostim; monoclonal antibody, human chorionic gonadotrophin; monophosphoryl lipid A+myobacterium cell wall sk; mopidamol; multiple drug resistance gene inhibitor; multiple tumor suppressor 1-based therapy; mustard anticancer agent; mycaperoxide B; mycobacterial



cell wall extract; myriaporone; N-acetyldinaline; N-substituted benzamides; nafarelin;  
nagrestip; naloxone+pentazocine; napavin; naphterpin; nartograstim; nedaplatin; nemorubicin;  
neridronic acid; neutral endopeptidase; nilutamide; nisamycin; nitric oxide modulators;  
nitroxide antioxidant; nitrullyn; O6-benzylguanine; octreotide; okicenone; oligonucleotides;  
onapristone; ondansetron; ondansetron; oracin; oral cytokine inducer; ormaplatin; osaterone;  
oxaliplatin; oxaunomycin; taxel; taxel analogues; taxel derivatives; palauamine;  
palmitoylrhizoxin; pamidronic acid; panaxytriol; panomifene; parabactin; pazelliptine;  
pegaspargase; peldesine; pentosan polysulfate sodium; pentostatin; pentozole; perflubron;  
perfosfamide; perillyl alcohol; phenazinomycin; phenylacetate; phosphatase inhibitors;  
picibanil; pilocarpine hydrochloride; pirarubicin; piritrexim; placetin A; placetin B;  
plasminogen activator inhibitor; platinum complex; platinum compounds; platinum-triamine  
complex; porfimer sodium; porfiromycin; prednisone; propyl bis-acridone; prostaglandin J2;  
proteasome inhibitors; protein A-based immune modulator; protein kinase C inhibitor; protein  
kinase C inhibitors, microalgal; protein tyrosine phosphatase inhibitors; purine nucleoside  
phosphorylase inhibitors; purpurins; pyrazoloacridine; pyridoxylated hemoglobin  
polyoxyethylene conjugate; raf antagonists; raltitrexed; ramosetron; ras farnesyl protein  
transferase inhibitors; ras inhibitors; ras-GAP inhibitor; retelliptine demethylated; rhenium Re  
186 etidronate; rhizoxin; ribozymes; RII retinamide; rogletimide; rohitukine; romurtide;  
roquinimex; rubiginone B1; ruboxyl; safingol; saintopin; SarCNU; sarcophytol A;  
sargramostim; Sdi 1 mimetics; semustine; senescence derived inhibitor 1; sense  
oligonucleotides; signal transduction inhibitors; signal transduction modulators; single chain  
antigen binding protein; sizofiran; sobuzoxane; sodium borocaptate; sodium phenylacetate;  
solverol; somatomedin binding protein; sonermin; sparfosic acid; spicamycin D;  
spiromustine; splenopentin; spongistatin 1; squalamine; stem cell inhibitor; stem-cell division  
inhibitors; stipiamide; stromelysin inhibitors; sulfinosine; superactive vasoactive intestinal

peptide antagonist; suradista; suramin; swainsonine; synthetic glycosaminoglycans; tallimustine; tamoxifen methiodide; tauromustine; tazarotene; tecogalan sodium; tegafur; tellurapyrylium; telomerase inhibitors; temoporfin; temozolomide; teniposide; tetrachlorodecaoxide; tetrazomine; thaliblastine; thiocoraline; thrombopoietin; thrombopoietin mimetic; thymalfasin; thymopoietin receptor agonist; thymotrinan; thyroid stimulating hormone; tin ethyl etiopurpurin; tirapazamine; titanocene bichloride; topsentin; toremifene; totipotent stem cell factor; translation inhibitors; tretinoin; triacetyluridine; triciribine; trimetrexate; triptorelin; tropisetron; turosteride; tyrosine kinase inhibitors; tyrphostins; UBC inhibitors; ubenimex; urogenital sinus-derived growth inhibitory factor; urokinase receptor antagonists; vapreotide; variolin B; vector system, erythrocyte gene therapy; velaresol; veramine; verdins; verteporfin; vinorelbine; vinxaltine; vitaxin; vorozole; zanoterone; zeniplatin; zilascorb; and zinostatin stimalamer. Preferred additional anti-cancer drugs are 5-fluorouracil and leucovorin. Additional cancer therapeutics include monoclonal antibodies such as rituximab, trastuzumab and cetuximab.

Reference to a chemotherapeutic agent herein applies to the chemotherapeutic agent or its derivatives and accordingly the invention contemplates and includes either of these embodiments (agent; agent or derivative(s)). "Derivatives" or "analogs" of a chemotherapeutic agent or other chemical moiety include, but are not limited to, compounds that are structurally similar to the chemotherapeutic agent or moiety or are in the same general chemical class as the chemotherapeutic agent or moiety. In some embodiments, the derivative or analog of the chemotherapeutic agent or moiety retains similar chemical and/or physical property (including, for example, functionality) of the chemotherapeutic agent or moiety.

The invention also relates to pharmaceutical or diagnostic compositions comprising the nanoparticles of the invention and a pharmaceutically acceptable carrier. The phrase "pharmaceutically acceptable carrier" is art recognized and includes a pharmaceutically

acceptable material, composition or vehicle, suitable for administering compounds used in the methods described herein to subjects, *e.g.*, mammals. The carriers include liquid or solid filler, diluent, excipient, solvent or encapsulating material, involved in carrying or transporting the subject agent from one organ, or portion of the body, to another organ, or portion of the body. Each carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not injurious to the patient. Some examples of materials which can serve as pharmaceutically acceptable carriers include: sugars, such as lactose, glucose and sucrose; starches, such as corn starch and potato starch; cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; powdered tragacanth; malt; gelatin; talc; excipients, such as cocoa butter and suppository waxes; oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; glycols, such as propylene glycol; polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; esters, such as ethyl oleate and ethyl laurate; agar; buffering agents, such as magnesium hydroxide and aluminum hydroxide; alginic acid; pyrogen-free water; isotonic saline; Ringer's solution; ethyl alcohol; phosphate buffer solutions; and other non-toxic compatible substances employed in pharmaceutical formulations. Suitable pharmaceutical carriers are described in Remington's Pharmaceutical Sciences, Mack Publishing Company, a standard reference text in this field.

### **Methods of Treatment**

The invention encompasses methods of treating or preventing diseases or disorders by administering to subjects in need thereof an effective amount of an activatable nanoparticle system described herein or a composition comprising same. Accordingly, a number of diseases or disorders are suitable for treatment according to the methods of the invention. Examples include, but are not limited to, Adenoma, Ageing, AIDS/ HIV, Alopecia, Alzheimer's disease, Anemia, Arthritis, Asthma, Atherosclerosis, Cancer, Cardiac conditions

or disease, Diabetes mellitus, Foodborne illness, Hemophilia A – E, Herpes, Huntington's disease, Hypertension, Headache, Influenza, Multiple Sclerosis, Myasthenia gravis, Neoplasm, Obesity, Osteoarthritis, Pancreatitis, Parkinson's disease, Pelvic inflammatory disease, Peritonitis, Periodontal disease, Rheumatoid arthritis, Sepsis, Sickle-cell disease, Teratoma, Ulcerative colitis, and Uveitis.

The methods of the invention further encompass diagnostics.

The methods may be practiced in an adjuvant setting. "Adjuvant setting" refers to a clinical setting in which, for example, an individual has had a history of a proliferative disease, particularly cancer, and generally (but not necessarily) been responsive to therapy, which includes, but is not limited to, surgery (such as surgical resection), radiotherapy, and chemotherapy. However, because of their history of the proliferative disease (such as cancer), these individuals are considered at risk of development of the disease. Treatment or administration in the "adjuvant setting" refers to a subsequent mode of treatment. The degree of risk (i.e., when an individual in the adjuvant setting is considered as "high risk" or "low risk") depends upon several factors, most usually the extent of disease when first treated. The methods provided herein may also be practiced in a neoadjuvant setting, i.e., the method may be carried out before the primary/definitive therapy. Thus, in some embodiments, the individual has previously been treated. In other embodiments, the individual has not previously been treated. In some embodiments, the treatment is a first line therapy.

### **Dosage**

Human dosage amounts of compositions of the activatable nanoparticle systems described herein can initially be determined by extrapolating from the amount of compound used in mice, as a skilled artisan recognizes it is routine in the art to modify the dosage for humans compared to animal models. In certain embodiments it is envisioned that the dosage may vary from between about 1 mg compound/Kg body weight to about 5000 mg

compound/Kg body weight; or from about 5 mg/Kg body weight to about 4000 mg/Kg body weight or from about 10 mg/Kg body weight to about 3000 mg/Kg body weight; or from about 50 mg/Kg body weight to about 2000 mg/Kg body weight; or from about 100 mg/Kg body weight to about 1000 mg/Kg body weight; or from about 150 mg/Kg body weight to about 500 mg/Kg body weight. In other embodiments this dose may be about 1, 5, 10, 25, 50, 75, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1050, 1100, 1150, 1200, 1250, 1300, 1350, 1400, 1450, 1500, 1600, 1700, 1800, 1900, 2000, 2500, 3000, 3500, 4000, 4500, 5000 mg/Kg body weight. In other embodiments, it is envisaged that higher doses may be used, such doses may be in the range of about 5 mg compound/Kg body to about 20 mg compound/Kg body. In other embodiments the doses may be about 8, 10, 12, 14, 16 or 18 mg/Kg body weight. Of course, this dosage amount may be adjusted upward or downward, as is routinely done in such treatment protocols, depending on the results of the initial clinical trials and the needs of a particular patient.

### **Methods of Delivery**

The activatable nanoparticle compositions described herein can be administered to an individual (such as human) via various routes, such as parenterally, including intravenous, intra-arterial, intraperitoneal, intrapulmonary, oral, inhalation, intravesicular, intramuscular, intra-tracheal, subcutaneous, intraocular, intrathecal, or transdermal. For example, the nanoparticle composition can be administered by inhalation to treat conditions of the respiratory tract. The composition can be used to treat respiratory conditions such as pulmonary fibrosis, broncheolitis obliterans, lung cancer, bronchoalveolar carcinoma, and the like. In some embodiments, the nanoparticle composition is administered intravenously. In some embodiments, the nanoparticle composition is administered orally.

The dosing frequency of the administration of the nanoparticle composition depends on the nature of the therapy and the particular disease being treated. For example, dosing

frequency may include, but is not limited to, once daily, twice daily, weekly without break; weekly, three out of four weeks; once every three weeks; once every two weeks; weekly, two out of three weeks.

The administration of nanoparticles may be carried out at a single dose or at a dose repeated once or several times after a certain time interval. The appropriate dosage varies according to various parameters, for example the individual treated or the mode of administration.

The dosing frequency of the nanoparticle composition or the nanoparticle composition and the second therapeutic agent may be adjusted over the course of the treatment, based on the judgment of the administering physician.

When administered separately, the nanoparticle composition and the second therapeutic agent can be administered at different dosing frequency or intervals. For example, the nanoparticle composition can be administered weekly, while a second agent can be administered more or less frequently. In some embodiments, sustained continuous release formulation of the nanoparticle and/or second agent may be used. Various formulations and devices for achieving sustained release are known in the art. The doses required for the nanoparticle composition and/or the second agent may (but not necessarily) be lower than what is normally required when each agent is administered alone. Thus, in some embodiments, a subtherapeutic amount of the drug in the nanoparticle composition and/or the second agent are administered. "Subtherapeutic amount" or "subtherapeutic level" refer to an amount that is less than the therapeutic amount, that is, less than the amount normally used when the drug in the nanoparticle composition and/or the second agent are administered alone. The reduction may be reflected in terms of the amount administered at a given administration and/or the amount administered over a given period of time (reduced frequency).

A combination of the administration configurations described herein can be used. The combination therapy methods described herein may be performed alone or in conjunction with another therapy, such as surgery, radiation, chemotherapy, immunotherapy, gene therapy, and the like. Additionally, a person having a greater risk of developing the disease to be treated may receive treatments to inhibit and/or delay the development of the disease.

The dose of nanoparticle composition will vary with the nature of the therapy and the particular disease being treated. The dose should be sufficient to effect a desirable response, such as a therapeutic or prophylactic response against a particular disease.

Appropriate doses will be established by persons skilled in the art of pharmaceutical dosing such as physicians.

In certain embodiments, the siRNAs can be administered as bolaamphiphiles.

Bolaamphiphiles have relatively low toxicities, long persistence in the blood stream, and most importantly, in aqueous conditions can form poly-cationic micelles thus, becoming amenable to association with siRNAs. Depending on the application, the extent of siRNA chemical protection, delivery efficiency, and further intracellular release can be varied by simply changing the type of bolaamphiphile used (see, e.g. Kim et al. Mol Ther Nucleic Acids. 2: e80, 2013, incorporated by reference in its entirety herein).

### **Kits**

The disclosure provides kits for the treatment or prevention of disease. In one embodiment, the kit includes a therapeutic or prophylactic composition containing an effective amount of an agent of the invention (e.g., NPs) in unit dosage form. In some embodiments, the kit comprises a sterile container which contains a therapeutic or prophylactic compound; such containers can be boxes, ampoules, bottles, vials, tubes, bags, pouches, blister-packs, or other suitable container forms known in the art. Such containers



can be made of plastic, glass, laminated paper, metal foil, or other materials suitable for holding medicaments.

If desired an agent of the disclosure is provided together with instructions for administering it to a subject having or at risk of developing a disease. The instructions will generally include information about the use of the composition for the treatment or prevention of the disease (e.g., neoplasia or viral infection). In other embodiments, the instructions include at least one of the following: description of the compound; dosage schedule and administration for treatment or prevention of the disease or symptoms thereof; precautions; warnings; indications; counter-indications; overdosage information; adverse reactions; animal pharmacology; clinical studies; and/or references. The instructions may be printed directly on the container (when present), or as a label applied to the container, or as a separate sheet, pamphlet, card, or folder supplied in or with the container.

#### **Recombinant Polypeptide Expression**

The practice of the present invention employs, unless otherwise indicated, conventional techniques of molecular biology (including recombinant techniques), microbiology, cell biology, biochemistry and immunology, which are well within the purview of the skilled artisan. Such techniques are explained fully in the literature, such as, “Molecular Cloning: A Laboratory Manual”, second edition (Sambrook, 1989); “Oligonucleotide Synthesis” (Gait, 1984); “Animal Cell Culture” (Freshney, 1987); “Methods in Enzymology” “Handbook of Experimental Immunology” (Weir, 1996); “Gene Transfer Vectors for Mammalian Cells” (Miller and Calos, 1987); “Current Protocols in Molecular Biology” (Ausubel, 1987); “PCR: The Polymerase Chain Reaction”, (Mullis, 1994); “Current Protocols in Immunology” (Coligan, 1991). These techniques are applicable to the production of the polynucleotides and polypeptides of the invention, and, as such, may be considered in

making and practicing the invention. Particularly useful techniques for particular embodiments will be discussed in the sections that follow.

The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the assay, screening, and therapeutic methods of the invention, and are not intended to limit the scope of what the inventors regard as their invention.

## **EXAMPLES**

### **Introduction**

RNA interference (RNAi) is a cellular process that occurs post-transcriptionally to regulate gene expression. This pathway can be mobilized through the foreign introduction of small interfering RNAs (siRNA) allowing for the regulation of genes that contribute to the diseased state. In order to simultaneously target several genes inside the same diseased cell with multiple siRNAs, those siRNAs need to be co-delivered in a controlled fashion. The field of therapeutic RNA nanotechnology aims to develop novel technologies facilitating this task through the introduction of programmable RNA-based nanoparticles amenable to functionalization with various therapeutics.

These nanoparticles demonstrate a precise and reproducible formulation, modularity, and programmability. However, for further biomedical applications, additional control over their specific intracellular activation is desired in order to minimize any unwanted interactions *in vivo*. The present invention recently introduced an approach that relies on the use of RNA-DNA hybrids for delivery of multiple split functionalities and their simultaneous intracellular activation (see Afonin, K. A. et al., Activation of different split functionalities on re-association of RNA-DNA hybrids. *Nature Nanotechnology*, 2013, 8, 296-304).

The methodology involved splitting the functional RNA entities (e.g., RNAi activators) into two non-functional RNA-DNA hybrids. Each DNA strand was designed to be slightly (~12-nt) longer than its RNA counterpart. Thus, upon the formation of the RNA-DNA hybrids, these duplexes would have ssDNA toeholds that were complementary to each other. The presence of ssDNA toeholds on the extremities of the hybrids allowed for their mutual recognition, re-association, and release of the functional RNA when they were in close proximity. This approach allowed an additional handle on the specificity of the site of action and conferred additional stability in vivo. See Id.

The inventors further demonstrated computationally (see Afonin, K. A., Computational and Experimental Studies of Reassociating RNA/DNA Hybrids Containing Split Functionalities. *Methods in Enzymology*, 2015, 553, 313-334) and experimentally (Afonin, K. A. et al., Co-transcriptional production of RNA-DNA hybrids for simultaneous release of multiple split functionalities. *Nucleic acids research* 2014, 42, 2085-2097) that multiple split RNA functionalities could be embedded in the same RNA-DNA hybrids, resulting in their simultaneous co-activation. This was achieved through the introduction, in a controlled fashion, of multiple functional entities, such as RNAi inducers (e.g., up-to seven at once), FRET fluorescence pairs for tracking of re-association in real time, and RNA aptamers for fluorescence response on re-association or specific cell targeting, just to name a few (Rogers, T. A. et al., Fluorescent monitoring of RNA assembly and processing using the split-spinach aptamer. *ACS Synthetic Biology*, 2015, 4, 162-166).

However, the number of functionalities activated at once was limited by the total length of the DNAs. In addition, the longer DNA duplexes that resulted from re-association became immunogenic (Afonin, K. A. et al., Co-transcriptional production of RNA-DNA hybrids for simultaneous release of multiple split functionalities. *Nucleic acids research* 2014, 42, 2085-2097). To overcome these limitations, individual hybrids were introduced as

the extremities of the compact RNA nanoparticles, nanorings and nanocubes, which were previously designed and characterized as programmable RNA nanoscaffolds. (See Afonin, K. A. et al., Design and self-assembly of siRNA-functionalized RNA nanoparticles for use in automated nanomedicine. *Nature Protocols*, 2011, 6, 2022-2034. Also see Afonin, K. A. et al., In vitro assembly of cubic RNA-based scaffolds designed in silico, *Nature Nanotechnology*, 2010, 5, 676-682. Also see Afonin, K. A. et al., Computational and experimental characterization of RNA cubic nanoscaffolds. *Methods* 2014, 67, 256-265. Also see Grabow, W. W et al. Self-assembling RNA nanorings based on RNAI/II inverse kissing complexes. *Nano Letters* 2011, 11, 878-887).

For all of the approaches described above, the ssDNA toeholds had to be specifically designed to avoid any internal secondary structures and any undesired interactions with the scaffold strands or split functions while triggering the re-association of the hybrids at physiological temperature (e.g., 37°C). These limitations would potentially require computer-aided sequence optimization for each new generation of hybrid nanoparticles.

By contrast, as described herein, the methodology disclosed in this invention schematically depicted in FIG. 1A relies solely on the use of RNA-DNA hybrids with ssRNA toeholds and demonstrates a significant simplification of all design principles in the existing technique. The overall size of the new nanoparticles amenable to intracellular activation becomes significantly smaller (e.g., up to 120 fewer nucleotides per nanoparticle) compared to designs relying on ssDNA toeholds. In addition, the current method using ssRNA toeholds allows better yields in the co-transcriptional assemblies of the hybrid nanoparticles. Also, a novel computation algorithm aiming at the prediction of multi-stranded hybrid secondary structures and their further re-association was developed to confirm the experimental results.

#### **Example 1: Rational design of RNA-DNA hybrids.**

As the proof of concept, the function of a Dicer Substrate RNA (DS RNA) was split and was designed to downregulate the production of green fluorescent protein (GFP) (Rose et al. Nucleic acids research 2005, 33, 4140-4156) that is stably expressed in model human breast cancer cells (MDA-MB231/GFP). The use of DS RNAs (as opposed to siRNAs) is required to ensure that once inside the cells, the individual hybrids will not be active in the RNAi pathway (Afonin et al. Nucleic acids research 2014, 42, 2085–2097). GFP DS RNAs were split between two RNA-DNA hybrids with the DNA strands being 8-, 6-, 4-, and 2-nts shorter than their corresponding complementary RNAs, thus, providing the ssRNA toeholds for further re-association. The hybrids containing the sense strand of DS RNA are referred as H<sub>sen</sub> and the hybrids containing the antisense strand are referred as H<sub>ant</sub>. A scheme explaining the re-association of new hybrids studied in this work is shown in FIG. 1A.

**Example 2: Re-association of RNA-DNA hybrids.**

Four sets of cognate RNA-DNA hybrids with different ssRNA toehold lengths (2-, 4-, 6-, and 8-nt) were prepared and tested in parallel (FIG. 1A-1C). The re-association of the hybrids was first assessed by native-PAGE experiments (FIG. 1C). The results show that the extent of re-association is dependent on the length of the ssRNA toeholds. In particular, only partial re-associations were observed for the hybrids with toeholds of 4-nt and less. *In silico* predictions based on a novel multi-strand secondary structure prediction approach confirmed these results.

To trace the re-association of hybrids in solution in real time, Förster resonance energy transfer (FRET) was measured. The kinetics of re-association were studied using fluorescently labeled (with Alexa 488 and Alexa 546) RNAs entering the composition of the different hybrids. When two fluorescently labeled hybrids are mixed and incubated at 37°C, their re-association brings Alexa 488 within the Förster radius ( $R_0 = 6.31$  nm) of Alexa 546 (FIG. 1C). Consequently, when excited at 460 nm, the emission of Alexa 488 significantly drops while the fluorescent signal of Alexa 546 increases (FIG. 1C). The results indicate strong dependence of the extent of re-association on the toehold lengths and confirm the previous observation.

### **Example 3: Re-association of RNA-DNA hybrids in human cells.**

The ability of the hybrids with ssRNA toeholds to enter and re-associate inside mammalian cells was assessed. Fluorescently labeled hybrids were co-transfected into human breast cancer cells and analyzed with confocal microscopy the next day (FIG. 1D) (Afonin et al ACS nano 2015, 9, 251-259; Afonin et al Nano letters 2014, 14, 5662-5671; Afonin et al Nature nanotechnology 2013, 8, 296-304). The samples were excited at 488 nm and the emission of Alexa546 was collected. To estimate the extent of intracellular FRET, Alexa546 sensitized emission was imaged as detailed in our previous work (Afonin et al Nature nanotechnology 2013, 8, 296-304). The FRET signal remaining upon bleed-through correction was calculated and is shown in blue (FIG. 1D, images 1+4 and 5). The ssRNA-toehold driven intracellular re-association of RNA-DNA hybrids was further confirmed by specific gene silencing experiments with human breast cancer cells stably expressing GFP. First, cells were co-transfected with only one hybrid at a time ( $H_{ant}$ ), and three days later, the level of eGFP expression was analyzed with fluorescence microscopy and flow cytometry. All experiments were repeated in triplicates, and results demonstrated no GFP silencing caused by the individual hybrids. However, when the same cells were co-transfected with

separately prepared complexes of Lipofectamine 2000 (L2K) and individual hybrids (H<sub>sen</sub>/L2K and H<sub>ant</sub>/L2K), significant GFP silencing was observed (FIG. 1E). It was hypothesized that the longer ssRNA toeholds would be positively correlated with a higher level of re-association and gene silencing. Interestingly, the extent of silencing was proportional to the ssRNA toehold lengths only at relatively low concentrations (1 nM) but not at the higher ones (30 nM). Even though the re-association was not detected for hybrids with 2-nts toeholds at 1  $\mu$ M concentrations (FIG. 1B-1C), significant gene silencing was observed for the same hybrids at 30 nM concentrations. This discrepancy can be explained by the use of the polycationic carrier (L2K) that electrostatically attracts high quantities of individual hybrids during the transfection and incubation step, and then releases them intracellularly in much smaller volumes (in endosomes), thus, providing a significant increase in the “local” concentration of cognate hybrid. To test the feasibility of this existing hypothesis, several re-association experiments at higher hybrid concentrations were carried; these showed that at high enough concentrations (5  $\mu$ M or higher), the re-association occurs even between the hybrids with 2-nts ssRNA toeholds.

To demonstrate the generality and the therapeutic potential of this approach, we tested two additional sets of hybrids designed against the full-length genomic HIV-1 RNA that also serves as the mRNA coding for the viral structural proteins and enzymes (Berkhout et al Antiviral research 2011, 92, 7-14.; Liu et al Molecular therapy : the journal of the American Society of Gene Therapy 2009, 17, 1712-1723; Low et al Molecular therapy : the journal of the American Society of Gene Therapy 2012, 20, 820-828). Gag and LDR hybrids targeted the sequences coding the capsid domain and the amino terminus of the matrix domain of Gag respectively (FIG. 2A-2B). Both hybrids were designed to have 8-nt ssRNA toeholds. Transfection of the individual hybrids showed no significant effect on relative virus release.

However, the co-transfection of cognate hybrids reduced the production of virus by 65% to 75% (FIG. 2C). The total amount of Gag was reduced on average by 65% with the reassociated hybrids, while the cellular expression levels of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were not affected.

**Example 4: Controlled activation of RNAi and by nanorings functionalized with RNA-DNA hybrids.**

To guarantee the simultaneous delivery and intracellular activation of multiple RNA-DNA hybrids, the previously characterized nanorings were employed (Grabow et al Nano letters 2011, 11, 878-887). Each nanoring was functionalized with six RNA-DNA hybrids of varying ssRNA toehold lengths (2-, 4-, 6-, and 8-nts in length). The toeholds were oriented towards the nanoring scaffolds to improve the stability of ssRNAs (exonuclease degradation). All nanoparticles were produced co-transcriptionally (Afonin et al Nature nanotechnology 2010, 5, 676-682; Afonin et al Nano letters 2012, 12, 5192-5195; Afonin et al Nucleic acids research 2012, 40, 2168-2180; Geary et al Science 2014, 345, 799-804), gel purified, recovered and further characterized. The relative yields of the co-transcriptionally produced nanoparticles with ssRNA toeholds were higher than the yields of the previously characterized nanoparticles (Afonin et al Nano letters 2014, 14, 5662-5671) containing ssDNA toeholds. All assemblies were confirmed by native-PAGE and their re-association was visualized using fluorescently labeled cognate hybrids (FIG. 3B). In agreement with previous results, the assemblies with 4- and 2-nts ssRNA toeholds demonstrated only limited re-association and the 6- and 8-nts toeholds were the most effective in forming the fully functionalized nanorings (with six DS RNAs). The formation of functional rings was further supported by cell transfection experiments (FIG. 3C).



**Example 5: Computational studies of RNA-DNA hybrid re-association.**

The estimated free energies of the non-re-associated hybrid state, the re-associated state and a quadruplex intermediate state are shown in FIG. 4A for different toehold lengths and for different concentrations. Interestingly, the computational thermodynamic predictions indicate that all toehold strand lengths should lead to re-association, because the re-associated state has a lower free energy compared to the RNA/DNA hybrids (FIG. 4A). Estimating, however, the free energy of activation of the transition state (a model of a quadruplex conformation consisting of non-re-associated RNA/DNA hybrid helices but bound cognate toeholds was used), suggests that hybrid sequences with toehold lengths of less than 6 nt are prevented from re-association because of an energetic barrier, possibly leading to kinetic frustration. Conversely, there is for high concentrations and toehold lengths greater 4 nt (according to the computational model), no kinetic barrier with respect to the model transition state (FIG. 4A-4B).

Furthermore, the computational structure predictions for the RNA nanoring scaffold sequences (FIG. 4C) correspond to a ring structure connected by kissing loop interactions. A secondary structure prediction of the functionalized nanoring sequences with cognate DNAs (FIG. 4D) shows the expected strand interactions with the exception of one kissing loop interaction.

The promise of RNA interference based therapeutics is made evident by the recent surge of biotechnological drug companies that pursue such therapies and their progression into human clinical trials. Recent achievements in RNA nanotechnology introduced nanoscaffolds (nanorings) with the potential for a broad use in biomedical applications (PCT/US10/38818, incorporated by reference in its entirety herein). As presented herein, besides functionalization with multiple short interfering RNAs for combinatorial RNA interference, these nanoscaffolds

also allow simultaneous embedment of assorted RNA aptamers, fluorescent dyes, proteins, as well as recently developed auto-recognizing RNA-DNA hybrids used to conditionally activate multiple split functionalities. These new constructs were extensively characterized and visualized in vitro, in cell culture and in vivo by various experimental techniques. The results revealed a higher detection sensitivity of diseased cells and significant increases in silencing efficiencies of targeted genes compared to the silencing caused by equal amounts of conventional siRNAs. Due to the combinatorial nature and relative engineering simplicity, these RNA nanoparticles are expected to be useful for various nanotechnological applications.

### **Methods**

The foregoing experiments were carried out with, but not limited to, the following methods and materials.

**RNA nanoring sequence design assemblies and native PAGE.** The detailed design and production of RNA strands entering the composition of nanorings functionalized with six siRNAs is comprehensively described elsewhere (Afonin et al Multifunctional RNA nanoparticles. Nano Letters 2014, 14, 5662-5671). The full list of RNA sequences used is available, and is shown below.

### ***Sequences***

Any suitable sequences of RNA and DNA may be obtain, designed, or otherwise provided by any means to prepare, manufacture, or otherwise assemble the nanoparticles of the invention. For example, RNA and DNA sequences used to assembly RNA-DNA hybrids containing split asymmetric 25/27mer Dicer substrate RNA (DS RNA) duplex designed against eGFP (Rose et al Nucleic acids research 2005, 33, 4140-4156). Exemplary sequences include:

DS RNA sense: 5' - pACCCUGAAGUUCAUCUGCACCACcg

sense labeled with Aexa-488: 5` - pACCCUGAAGUUCAUCUGCACCACcg-AI488

DNA for sense(8nts RNA toehold) 5` - CAGATGAACTTCAGGGTca

DNA for sense(6nts RNA toehold) 5` - TGCAGATGAACTTCAGGGTca

DNA for sense(4nts RNA toehold) 5` - GGTGCAGATGAACTTCAGGGTca

DNA for sense(2nts RNA toehold) 5` - GTGGTGCAGATGAACTTCAGGGTca

DS RNA antisense: 5` - CGGUGGUGCAGAUGAACUUCAGGGUCA antisense labeled with Alexa546: 5`-AI546-CGGUGGUGCAGAUGAACUUCAGGGUCA

DNA for ant(8nts RNA toehold) 5` - TGACCCTGAAGTTCATCTG

DNA for ant(6nts RNA toehold) 5` - TGACCCTGAAGTTCATCTGCA

DNA for ant(4nts RNA toehold) 5` - TGACCCTGAAGTTCATCTGCACC

DNA for ant(2nts RNA toehold) 5` - TGACCCTGAAGTTCATCTGCACCAC

RNA and DNA sequences used to assembly RNA-DNA hybrids containing split asymmetric DSRNA duplex designed against HIV (Berkhout et al Antiviral research 2011, 92, 7-14; Liu et al Molecular therapy : The Journal of the American Society of Gene Therapy 2009, 17, 1712-1723; Low et al Molecular Therapy : the Journal of the American Society of Gene Therapy 2012, 20, 820-828). The names of corresponding DS RNAs are indicated for each DS RNA: Capsid (GAG) and Primer Binding Site – Matrix (LDR). Exemplary sequences include:

#### **Capsid (Gag)**

Sense:

5'-pGAAGAAAUGAUGACAGCAUUUCAGG

DNA for GAG sense (RNA toehold):

5'-GCTGTCATCATTTCTTCTT

Antisense:

5'-CCUGAAAUGCUGUCAUCAUUUCUUCUU

DNA for GAG ant (RNA toehold):

5'-AAGAAGAAATGATGACAGC

**Primer Binding Site – Matrix (LDR)**

Sense:

5'-pGGAGAGAGAUGGGUGCGAGUUCGUC

DNA for LDR sense (RNA toehold):

5'-CGCACCCATCTCTCTCCTT

Antisense:

5'-GACGGACUCGCACCCAUCUCUCUCCUU

DNA for LDR ant (RNA toehold):

5'-AAGGAGAGAGATGGGTGCG

RNA nanorings 3'-side functionalized with DS RNA antisenses against Green  
Fluorescent Protein (Afonin et al Nano Letters 2014, 14, 5662-5671).

A: 5'-

GGGAACCGUCCACUGGUUCCCGCUACGAGAGCCUGCCUCGUAGCUUCGGUGGUGC  
AGAUGAACUUCAGGGUCA

B: 5'-

GGGAACCGCAGGCUGGUUCCCGCUACGAGAGAACGCCUCGUAGCUUCGGUGGUGC  
AGAUGAACUUCAGGGUCA

C: 5'-

GGGAACCGCGUUCUGGUUCCCGCUACGAGACGUCUCCUCGUAGCUUCGGUGGUGC  
AGAUGAACUUCAGGGUCA

D: 5'-

GGGAACCGAGACGUGGUUCCCGCUACGAGUCGUGGUCUCGUAGCUUCGGUGGUGC  
AGAUGAACUUCAGGGUCA

E: 5'-

GGGAACCACCACGAGGUUCCCGCUACGAGAACCAUCCUCGUAGCUUCGGUGGUGC  
AGAUGAACUUCAGGGUCA

F: 5'-

GGGAACCGAUGGUUGGUUCCCGCUACGAGAGUGGACCUCGUAGCUUCGGUGGUG  
CAGAUGAACUUCAGGGUCA

RNA molecules were purchased (from Integrated DNA Technologies, Inc., for short RNAs, e.g., siRNAs and/or DsiRNAs) or prepared by transcription of PCR amplified DNA templates; synthetic DNA molecules coding for the sequence of the designed RNA were purchased already amplified by PCR using primers containing the T7 RNA polymerase promoter (see PCT/US2013/058492, filed September 6, 2013, incorporated by reference in its entirety herein). PCR products were purified using the QiaQuick PCR purification kit and RNA molecules were prepared enzymatically by *in vitro* transcription using T7 RNA polymerase. For the visualization of assembled RNA NPs quality control experiments, [<sup>32</sup>P]Cp labeled RNA molecules were used (T4 RNA ligase is used to label the 3'-ends of RNA molecules by attaching [<sup>32</sup>P]Cp<sup>19</sup>). In the case of the initial radiolabel native-PAGE assays, radiolabeled RNA scaffold strand was mixed with concatenated strands individually followed by the assembly protocol<sup>4</sup>. For

dicing functional control experiments, RNA molecules were co-transcriptionally  $\alpha$ [P<sup>32</sup>]-ATP body-labeled. Native PAGE experiments were performed as described<sup>20</sup>. Typically, assembly experiments reported were analyzed at 10 °C on 7% (29:1) native polyacrylamide gels in the presence of 89 mM Tris-borate, pH 8.3, 2 mM Mg(OAc)<sub>2</sub>. A Hitachi FMBIO II Multi-View Imager was used to visualize SYBR Gold stained R/DNA hybrids.

### **RNA/DNA nanoparticles and hybrid assemblies and native PAGE.**

The designing principles and production of RNA strands entering the composition of the nanorings functionalized with six siRNAs is comprehensively described elsewhere (Afonin et al Multifunctional RNA nanoparticles. Nano letters 2014, 14, 5662-5671). RNA-DNA hybrids were assembled as detailed elsewhere (Afonin et al, Activation of different split functionalities on re-association of RNA-DNA hybrids. Nature nanotechnology 2013, 8, 296–304) and RNA/DNA nanorings were produced co-transcriptionally (Afonin et al, Co-transcriptional assembly of chemically modified RNA nanoparticles functionalized with siRNAs. Nano letters 2012, 12, 5192-5195). Briefly, synthetic DNA molecules coding for the sequence of the designed RNA were purchased and amplified by PCR using primers containing the T7 RNA polymerase promoter. Corresponding DNA templates were mixed together with ssDNA required for hybrid formations and RNA-DNA nanoparticles were produced enzymatically by *in vitro* run-off transcription using wildtype T7 RNA polymerase. Transcription was performed in 80 mM HEPES-KOH, pH 7.5; 2 mM spermidine; 50 mM DTT; 25 mM MgCl<sub>2</sub>; 1 mM NTPs; 0.2  $\mu$ M of DNA templates, 50  $\mu$ M of ssDNA for hybrids; and T7 RNA polymerase. The resulting co-transcriptionally assembled nanorings functionalized with six hybrids were purified by native-PAGE and further analyzed. Typically, all assembly experiments were analyzed at 10°C on 7% (37.5:1) native polyacrylamide gels in the presence of 89 mM Tris-borate, pH 8.3 and 2 mM



Mg(OAc)<sub>2</sub>. ChemiDoc MP System was used to visualize fluorescently labeled (Alexa 488 and Alexa 546) and EtBr stained RNA-, RNA-DNA- and DNA-RNA-based assemblies.

#### **FRET studies.**

Re-association of cognate RNA-DNA hybrids *in vitro* was tracked with FRET measurements using a FluoroMax3 (Jobin-Yvon, Horiba). All fluorescently labeled RNAs are presented in Supporting Information (Fluorescently labeled molecules). For all the experiments, the excitation wavelength was set at 460 nm, and the excitation and emission slit widths were set at 2 nm.

Alexa 488 labeled constructs were first incubated for two minutes at 37°C, and then Alexa 546 labeled constructs were added. Upon excitation at 460 nm, the emissions at 520 nm and 570 nm were recorded simultaneously every 30 seconds to follow the process of re-association through FRET measurements. This experiment was also carried out with hybrids individually pre-incubated with L2K (Invitrogen) in the amounts relevant for the transfection conditions. *In vitro* fluorescent experiments show no re-association of these complexes in solution thus, the re-association occurs only in cells.

#### **Transfection of human breast cancer cells MDA-MB-231.**

MDA-MB-231 cells either with or without eGFP were grown in a 5% CO<sub>2</sub> incubator in DMEM (Gibco BRL) supplemented with 10% FBS and penicillin-streptomycin. All transfections in this project were performed using L2K. 50X solutions of hybrids were individually pre-incubated at room temperature with L2K for 30 minutes. RNA-DNA nanorings were co-transfected with their cognate RNA-DNA hybrids (at six-fold higher concentrations). To avoid re-association in media, cognate RNA-DNA hybrids were pre-incubated with L2K separately. Prior to each transfection, the cell media was swapped with OPTI-MEM mixed with prepared 50X of hybrids/L2K complexes to the final concentration of 1X. The cells were

incubated for 4 hours followed by the media change (D-MEM, 10% FBS, 1% pen-strep) (Afonin et al, Triggering of RNA Interference with RNA-RNA, RNA-DNA, and DNA-RNA Nanoparticles. *ACS nano* **2015**, 9, 251-259 and Afonin et al, Multifunctional RNA nanoparticles. *Nano letters* **2014**, 14, 5662-5671 and Afonin et al, Activation of different split functionalities on re-association of RNA-DNA hybrids. *Nature nanotechnology* 2013, 8, 296-304).

### **Confocal microscopy.**

The intracellular re-association of RNA-DNA hybrids was assessed through FRET Afonin et al, Triggering of RNA Interference with RNA-RNA, RNA-DNA, and DNA-RNA Nanoparticles. *ACS nano* **2015**, 9, 251-259 and Afonin et al, Multifunctional RNA nanoparticles. *Nano letters* **2014**, 14, 5662-5671 and Afonin et al, Activation of different split functionalities on re-association of RNA-DNA hybrids. *Nature nanotechnology* 2013, 8, 296-304) . All measurements were performed using a LSM 710 confocal microscope (Carl Zeiss) with a 63x, 1.4 NA magnification lens. All images were taken with a pinhole adjusted to 1 airy unit. Fluorescently labeled hybrid nanoparticles and cognate hybrids were individually pre-incubated with L2K and co-transfected into cells. On the next day, the samples were fixed by incubation in 4% paraformaldehyde for 20 minutes at room temperature. Images of the cells were then taken to assess the appearance of FRET within the sample. For Alexa 488 imaging, the 488 nm line of an Argon laser was used as excitation and the emission was collected between 493 and 557 nm. For Alexa 546 imaging, a DPSS 561 laser was used for excitation, and emission was collected between 566 and 680 nm. In order to evaluate the sensitized emission through FRET, images were taken exciting the sample with the 488 nm line and collecting emission between 566 and 680 nm. Because of spectral overlap, the FRET signal is contaminated by donor emission into the acceptor channel and by the excitation of acceptor



molecules by the donor excitation wavelength. This bleed-through was assessed via measurements performed with samples transfected with individual dyes and mathematically removed from the images of FRET.

#### **Flow cytometry experiments.**

MDA-MB-231 cells expressing GFP were grown in 12-well plates ( $1.0 \times 10^5$  cells per well) and lifted with a cell dissociation buffer (Gibco BRL). The level of GFP was measured by fluorescence-activated cell sorting (FACS) analysis on a FACScalibur flow cytometer (BD Bioscience) (Baugh et al, *Journal of molecular biology* **2000**, 301, 117-128; Gupta et al, *Journal of controlled release : official journal of the Controlled Release Society* **2015**, 213, 142-151; Gupta et al, *Nanomedicine* **2015**, 1-14).

At least 20,000 events were collected and analyzed using the CellQuest software. For statistical analysis, the geometric mean fluorescence intensity (gMFI) and standard error of the mean (SEM) were calculated and plotted (Afonin et al, Triggering of RNA Interference with RNA-RNA, RNA-DNA, and DNA-RNA Nanoparticles. *ACS nano* **2015**, 9, 251-259 and Afonin et al, Multifunctional RNA nanoparticles. *Nano letters* **2014**, 14, 5662-5671).

#### **HIV-1 experiments.**

HeLa cells were cultured in a 24-well plate, in a 5% CO<sub>2</sub> incubator, in antibiotic-free DMEM (Gibco BRL) supplemented with 5% FBS (Atlanta biologicals). Nucleic acids and L2K were mixed at concentrations higher than recommended by the manufacturer; for each well, 0.66  $\mu$ g HIV-1 infectious clone pNL4-3 (Adachi et al, *Journal of virology* 1986, 59, 284-291), 0.05  $\mu$ g gaussia luciferase reporter plasmid pGLuc (NEB) and either 2.5 or 10 pmol of RNA/DNA hybrids, were combined in 15  $\mu$ l Optimem. 2.4  $\mu$ l L2K was mixed with 15  $\mu$ l Optimem and incubated for 5 minutes before combining with the nucleic acids. To avoid re-association in

media, cognate RNA-DNA hybrids were pre-incubated with L2K separately. The transfection mix was incubated for 30 minutes at room temperature then added dropwise to cells. After incubation for 8 hours, the media were removed and replaced with DMEM supplemented with 5% FBS, 100 U/ml penicillin, 100 jtg/ml streptomycin, and 2 mM L-glutamine (Gibco BRL). After 48 hours, supernatant was collected and supplemented with 0.5% Triton X-100 to inactivate infectious HIV-1 particles. Samples of supernatant were assayed for gaussia luciferase activity, as a reporter for transfection efficiency and toxicity, using Biolux (NEB) following the manufacturer's instructions; and for reverse transcriptase (RT), a component of the HIV-1 virion, as described previously<sup>48</sup>. Virus release was calculated as the ratio of RT to luciferase signal. Cells were also harvested in 2x Laemmli buffer (20% glycerol, 4% SDS, 0.01% bromophenol blue, 700 mM  $\beta$ -mercaptoethanol, 100 mM Tris pH 6.8) and probed by western blotting with HIV immunoglobulin (NIH AIDS Reagent Program Catalog #3957) and monoclonal mouse anti-GAPDH (clone 6C5 – Santa Cruz), and species-specific horseradish peroxidase-conjugated secondary antibodies (Thermo Scientific). Bands were revealed using chemiluminescence and the signal was recorded using a Chemidoc XRS+ system (Biorad). Band volumes were determined using Image Lab software (Biorad); to determine Gag expression, volumes for p55, p41 and CA were summed, and then divided by the corresponding GAPDH volume.

#### **In silico predictions of RNA-DNA hybrid re-association.**

In the novel computational approach (, an estimated free energy of a large variety of secondary structure states of the four involved sense and antisense RNA and DNA strands is computed. The free energy contributions of base pairing are based on base pair stacking energies for RNA/RNA, DNA/DNA and RNA/DNA interactions. Entropy changes with respect to the unfolded state are estimated using a geometric distance-based model. In this approach, for each

pair of nucleotides, maximally attainable residue-residue distances are computed for each examined secondary structure state. Each formed based pair can lead to a reduction of these maximally extended conformations and thus to a reduction in entropy. This distance-based approach is a general model for estimating entropy contributions that is not limited by the number of involved nucleotide strands and is applicable to pseudoknotted structures (Afonin et al, Computational and Experimental Studies of Reassociating RNA/DNA Hybrids Containing Split Functionalities. *Methods in enzymology* 2015, 553, 313-334). From this list of states, concentrations of the RNA/DNA hybrids, the re-associated RNA and DNA duplexes as well as a variety of intermediate states can be estimated.

The computational approach also identifies pairs of secondary structures that have a different strand connectivity and differ by only one additionally placed helix. The minimum energetic cost to place one additional helix to transition from one inter-strand connectivity to a different inter-strand connectivity is used to estimate the free energy of activation. For the case of RNA/DNA hybrids it was observed that the estimated free energy of activation (going from the non-re-associated state to an intermediate complex consisting of four strands) can lead to a qualitative prediction where the RNA/DNA hybrids can readily re-associate but are kinetically trapped due to short toehold lengths (leading to a high free energy of activation of the transition state).

The free energy of activation from the state of RNA/DNA hybrid duplexes to the quadruplex intermediate state is estimated by estimating the free energy change of the formation of the first duplex between the cognate toehold regions (without unfolding of any bases pairs of the RNA/DNA hybrid start state). In other words, for an RNA/DNA hybrid structure with 6nt toeholds, the free energy change of forming a 6nt helix between the cognate toehold regions of

the non-reassociated RNA/DNA hybrids strands is estimated as shown in FIG. 4B for different toehold lengths and strand concentrations. Other strand configurations (such as complexes involving 3 strands) were ruled out as important transition states because they are predicted to have unfavorable free energies.

The algorithm and computational method is also used to predict RNA secondary structures. A complete enumeration of all secondary structures is not feasible for longer nucleotide sequences because of a combinatorial explosion of possible secondary structures. Because of that, the algorithm performs an enumeration of secondary structures that are generated by choosing helices from a set of “core helices”. Those “core helices” are defined as helices that consist of at least 6 base pairs and that cannot be extended further on either end by Watson-Crick or GU base pairs.

#### **Representation of secondary structures.**

Each secondary structure is internally represented by a matrix of maximum and minimum distances between nucleotides. For nucleotide pairs of single-stranded regions of the same strand, their maximum distance is computed as the product of the number of separating nucleotides times a maximum distance per nucleotide. If both nucleotides are part of the same base pair, their maximum and minimum distances are set to a constant value. If two nucleotides belong to different nucleotide strands without inter-strand base pairing, their maximum distance is given through the inverse of the strand concentrations. For each folded base pair, all maximum and minimum nucleotide-nucleotide distances are updated to fulfill these rules. The maximum and minimum distances between residues define an estimated volume of conformational freedom that is used to compute the conformational entropy of a secondary structure. Importantly, the approach provides estimated entropy contributions for potentially pseudoknotted structures

consisting of multiple strands. Free energy contributions of nucleotide base pairing are computed using base pair stacking parameters for RNA/RNA, DNA/DNA and RNA/DNA interactions (Sugimoto et al, *Biochemistry* **1995**, 34, 11211; Mathews et al, *J Mol Biol* **1999**, 288, 911; SantaLucia et al, *Proc Natl Acad Sci U S A* **1998**, 95, 1460). Because this matrix data structure in “distance space” is memory-intensive, another more compact representation is utilized in form of a vector of integer numbers that indicate which of the core helices are part of the structure. A “0” at position  $i$  in that integer vector indicates that core helix  $i$  is not part of the structure; a “1” at position  $i$  indicates that a core helix  $i$  is in its full length present in the secondary structure. Indices greater than one describe different partially placed variants of a helix. The matrix representation of secondary structures facilitates entropy estimations, while the integer vector representation is memory-efficient. The program is able to inter-convert between the distance matrix representation and the integer vector representations thus making the distance matrix available only when needed.

#### **Algorithm.**

Briefly, this folding algorithm works as follows: Two priority queues A and B for storing free energy-ranked secondary structures are generated. To prevent the combinatorial explosion of the computational search, the implementation of the priority queue data structure is such that it automatically deletes the lowest-priority elements, if the number of stored elements exceeds a specified maximum size (this maximum size is set to 1000 elements). The two priority queues hold the integer vector representations of the partially folded nucleic acid complexes such that the contained structures are at any given time sorted by the estimated free energy of folding. The algorithm starts by generating an array of “core helices” (defined above) that is sorted in order of free energy. In addition, a second array of non-core “short helices” with lengths from 3 to 5 base



pairs is generated. Next, the integer vector representation of an unfolded structure (containing zero base pairs) is added to priority queue A. If there are  $n$  core helices, the search consists of  $n$  rounds. In the first round, the top-ranking element of queue A (which happens to be the previously added unfolded structure) is removed from queue A. Four variants of that structure are generated and added to queue B. These four variants are: i) core helix 1 being unplaced, ii) core helix 1 fully placed, iii) and iv) core helix 1 placed to half its length from either end. Helix candidates can be shortened in order to not conflict with already placed helices. Structures in which a helix cannot be placed are not stored. In other words, in round 1 the decision is made to what extent core helix 1 is placed. For each structure composed of a combination of core helices, another structural variant is generated by placing the previously generated short helices in the order of free energy, leading for the first round to up to  $4 \times 2 = 8$  stored structural variants. In the first round, queue B has the role of a “receiving” queue, and queue A has the role of “producing” queue. In the second round, queue A becomes the receiving queue and queue B the producing queue. For a general round  $i$ , and for each structure removed from the producing queue, the different variants with respect to the placement of core helix  $i$  are generated and the resulting secondary structures are added to the receiving queue (one version without additional small helices and one version with additional small helices). This process is continued until the producing queue is empty and all core helices have been explored.

In this fashion, all combinations of core helices are searched quasi-exhaustively while placing additional small helices in order of free energy for each examined combination of core helices. Helices can be pseudoknotted with the constraint that each helix can be non-nested with respect to at most one other helix. This approach thus combines entropy estimations and a

tunable search strategy that avoids redundant structure evaluations and is thus applicable (and used) for estimating the partition function.

### **Equivalents**

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

### **Incorporation by Reference**

Each of the applications and patents cited in this text, as well as each document or reference cited in each of the applications and patents (including during the prosecution of each issued patent; "application cited documents"), and each of the PCT and foreign applications or patents corresponding to and/or claiming priority from any of these applications and patents, and each of the documents cited or referenced in each of the application cited documents, are hereby expressly incorporated herein by reference. More generally, documents or references are cited in this text, either in a Reference List before the claims, or in the text itself; and, each of these documents or references ("herein-cited references"), as well as each document or reference cited in each of the herein-cited references (including any manufacturer's specifications, instructions, etc.), is hereby expressly incorporated herein by reference.

### **References**

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**What is claimed is:**

1. An activatable nanoparticle system comprising one or more split functionalities comprising a first inactive nanoparticle comprising a first set of DNA and/or RNA strands and a first ssRNA toehold and a second inactive nanoparticle comprising a second set of DNA and/or RNA strands and a second ssRNA toehold, wherein the strands of the first inactive nanoparticle are the reverse complements of the strands of the second inactive nanoparticle, wherein the first and second inactive nanoparticles are capable of undergoing reassociation of their strands to produce one or more functionalities, and wherein the reassociation of strands is triggered by the interaction of the first and second ssRNA toeholds.
2. The activatable nanoparticle system of claim 1, wherein the one or more split functionalities is selected from the group consisting split transcription, split aptamer, split optical response, and split Dicer substrate.
3. The activatable nanoparticle system of claim 1, wherein the ssRNA toeholds are 2, 4, 6, 8, 10, or 12 nucleotides.
4. The activatable nanoparticle system of claim 1, wherein the ssRNA toeholds are at least 4 nucleotides.
5. The activatable nanoparticle system of claim 1, wherein the ssRNA toeholds impart greater stability, smaller size, and greater production yields by run-off transcription as compared to nanoparticles with ssDNA toeholds.

6. The activatable nanoparticle system of claim 1, wherein the first or second nanoparticle is a nanoring, nanotube, or nanocube comprising one or more hybrid duplex arms comprising the first or second ssRNA toehold.

7. The activatable nanoparticle system of claim 1, wherein the first or second nanoparticle is an RNA/DNA duplex comprising the first or second ssRNA toehold.

8. The activatable nanoparticle system of claim 2, wherein the split Dicer substrate inhibits a target gene.

9. A method of triggering one or more functionalities in a cell comprising administering a therapeutically effective amount of the activatable nanoparticle system of claim 1.

10. A method of triggering one or more functionalities in a cell comprising:

- (a) administering an effective amount of a first inactive nanoparticle comprising a first set of DNA and/or RNA strands and a first ssRNA toehold;
- (b) administering an effective amount of a second inactive nanoparticle comprising a second set of DNA and/or RNA strands and a second ssRNA toehold;

wherein the strands of the first inactive nanoparticle are the reverse complements of the strands of the second inactive nanoparticle,

wherein the first and second inactive nanoparticles are capable of undergoing reassociation of their strands to produce one or more functionalities, and

wherein the reassociation of strands of the first and second nanoparticles is triggered by the interaction of the first and second ssRNA toeholds.

11. The method of triggering one or more functionalities of claim 10, wherein the one or more functionalities is selected from the group consisting transcription, aptamer, optical response, and Dicer substrate.

12. The method of triggering one or more functionalities of claim 10, wherein the ssRNA toeholds are 2, 4, 6, 8, 10, or 12 nucleotides.

13. The method of triggering one or more functionalities of claim 10, wherein the ssRNA toeholds are at least 4 nucleotides.

14. The method of triggering one or more functionalities of claim 10, wherein the ssRNA toeholds impart greater stability, smaller size, and greater production yields by run-off transcription as compared to nanoparticles with ssDNA toeholds.

15. The method of triggering one or more functionalities of claim 10, wherein the first or second nanoparticle is a nanoring, nanotube, or nanocube comprising one or more hybrid duplex arms comprising the first or second ssRNA toehold.

16. The method of triggering one or more functionalities of claim 10, wherein the first or second nanoparticle is an RNA/DNA duplex comprising the first or second ssRNA toehold.

17. The method of triggering one or more functionalities of claim 11, wherein the split Dicer substrate inhibits a target gene.

18. A method of inhibiting a target gene in a cell comprising:

(a) administering an effective amount of a first inactive nanoparticle comprising a first set of DNA and/or RNA strands and a first ssRNA toehold;

(b) administering an effective amount of a second inactive nanoparticle comprising a second set of DNA and/or RNA strands and a second ssRNA toehold;

wherein the strands of the first inactive nanoparticle are the reverse complements of the strands of the second inactive nanoparticle,

wherein the first and second inactive nanoparticles are capable of undergoing reassociation of their strands to produce one or more functionalities which inhibit a target gene in the cell, and

wherein the reassociation of strands of the first and second nanoparticles is triggered by the interaction of the first and second ssRNA toeholds.

19. The method of claim 18, wherein the one or more functionalities is selected from the group consisting transcription, aptamer, optical response, and Dicer substrate.

20. The method of claim 18, wherein the ssRNA toeholds are 2, 4, 6, 8, 10, or 12 nucleotides.



21. The method of claim 18, wherein the ssRNA toeholds are at least 4 nucleotides.
22. The method of claim 18, wherein the ssRNA toeholds impart greater stability, smaller size, and greater production yields by run-off transcription as compared to nanoparticles with ssDNA toeholds.
23. The method of claim 18, wherein the first or second nanoparticle is a nanoring, nanotube, or nanocube comprising one or more hybrid duplex arms comprising the first or second ssRNA toehold.
24. The method of claim 18, wherein the first or second nanoparticle is an RNA/DNA duplex comprising the first or second ssRNA toehold.
25. The method of claim 18, wherein the split Dicer substrate inhibits the target gene.



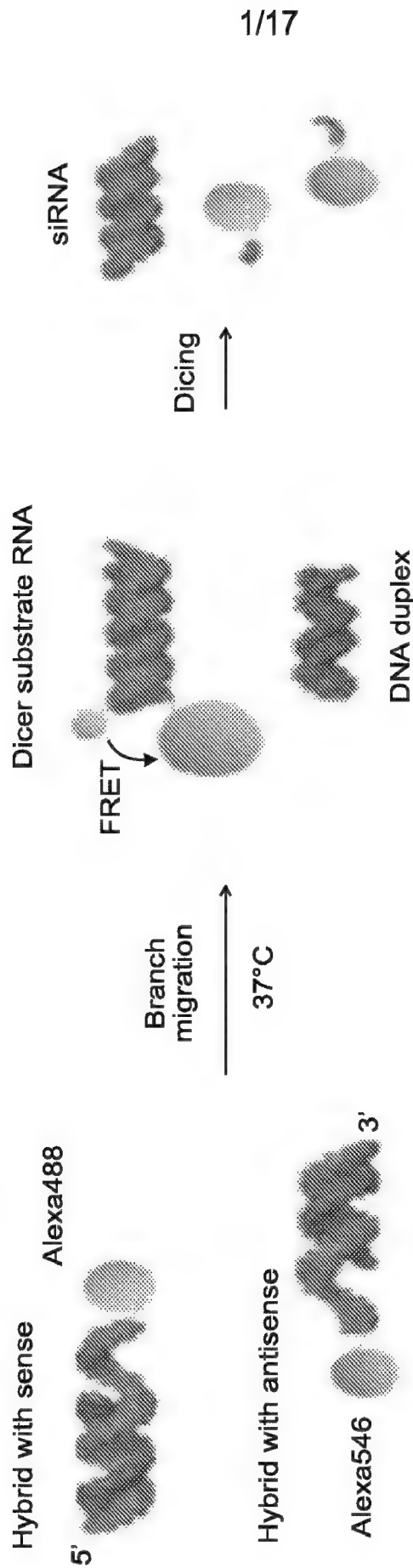


FIG. 1A

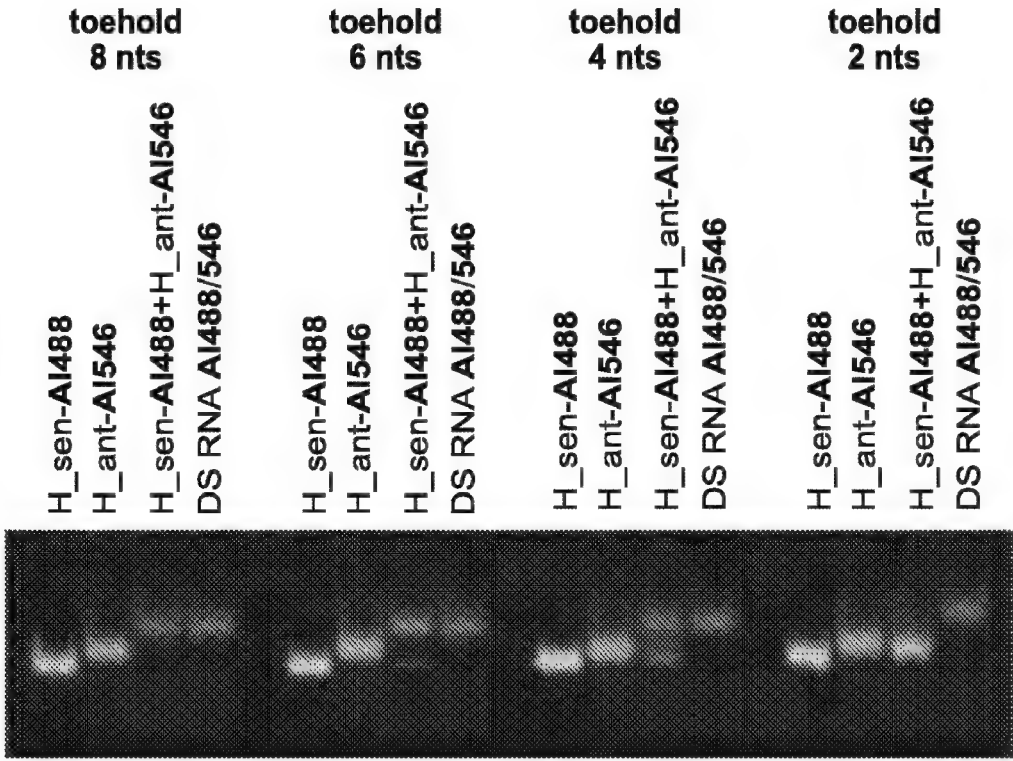


FIG. 1B

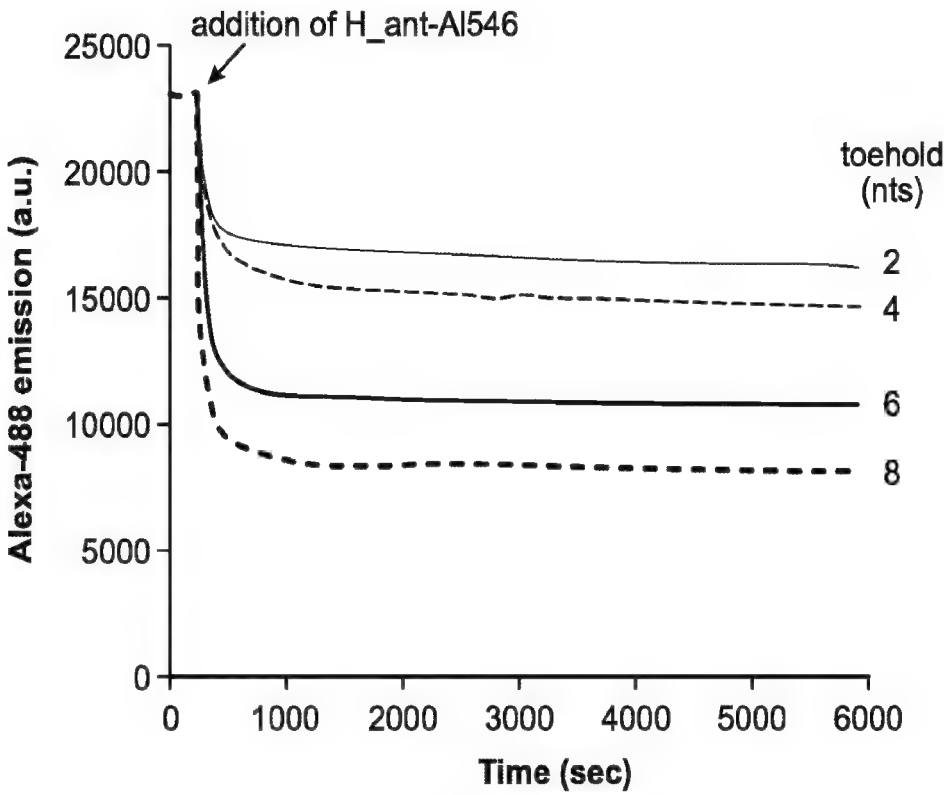


FIG. 1C

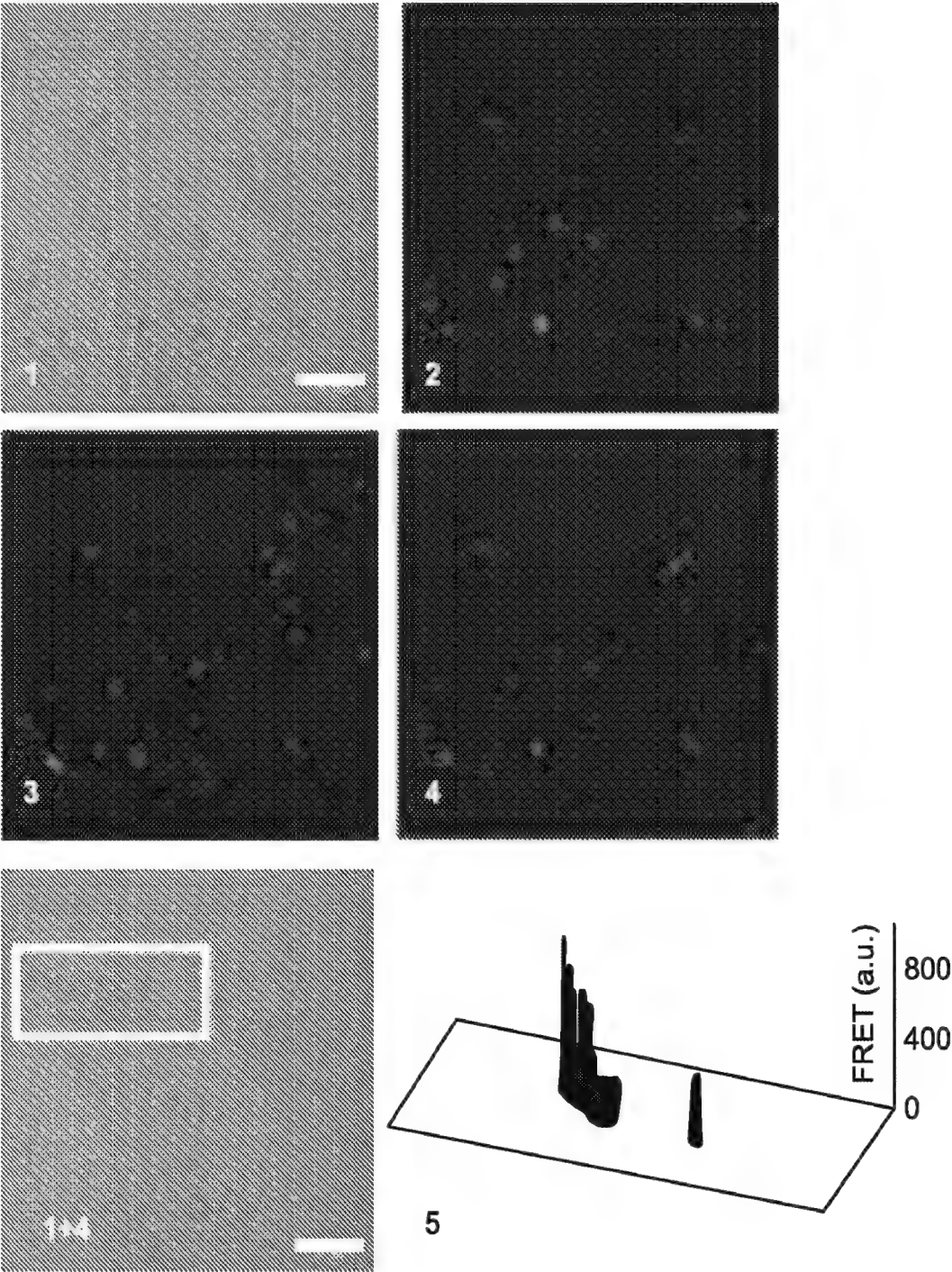


FIG. 1D



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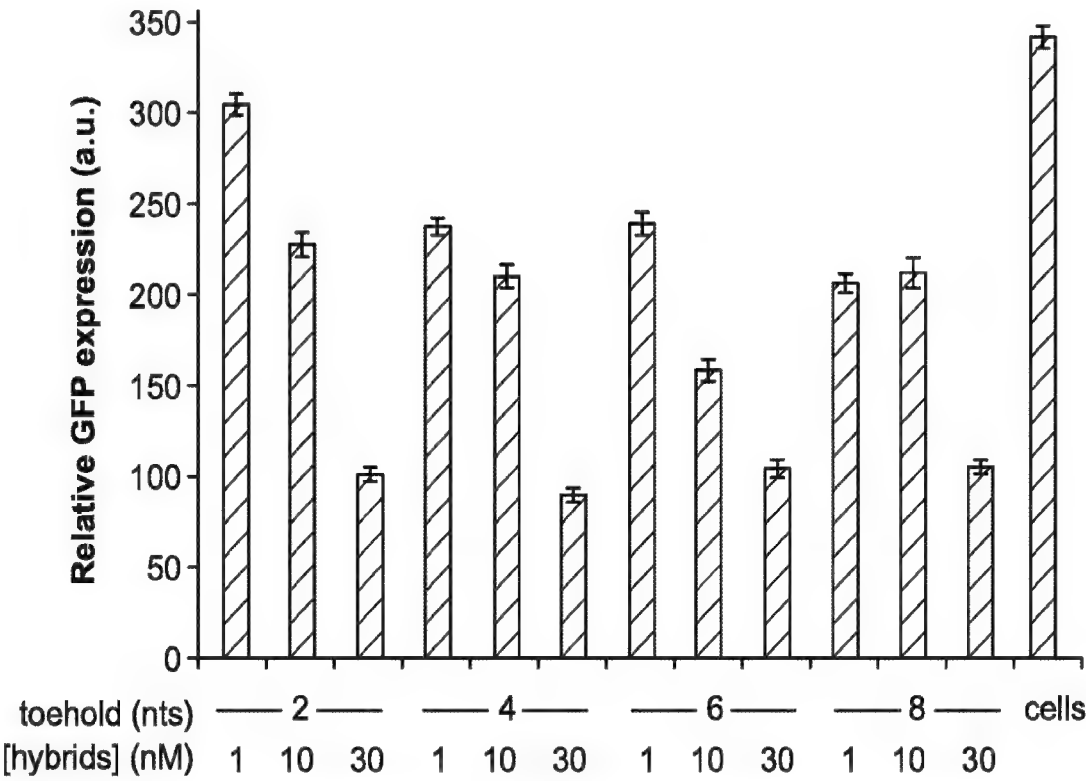


FIG. 1E

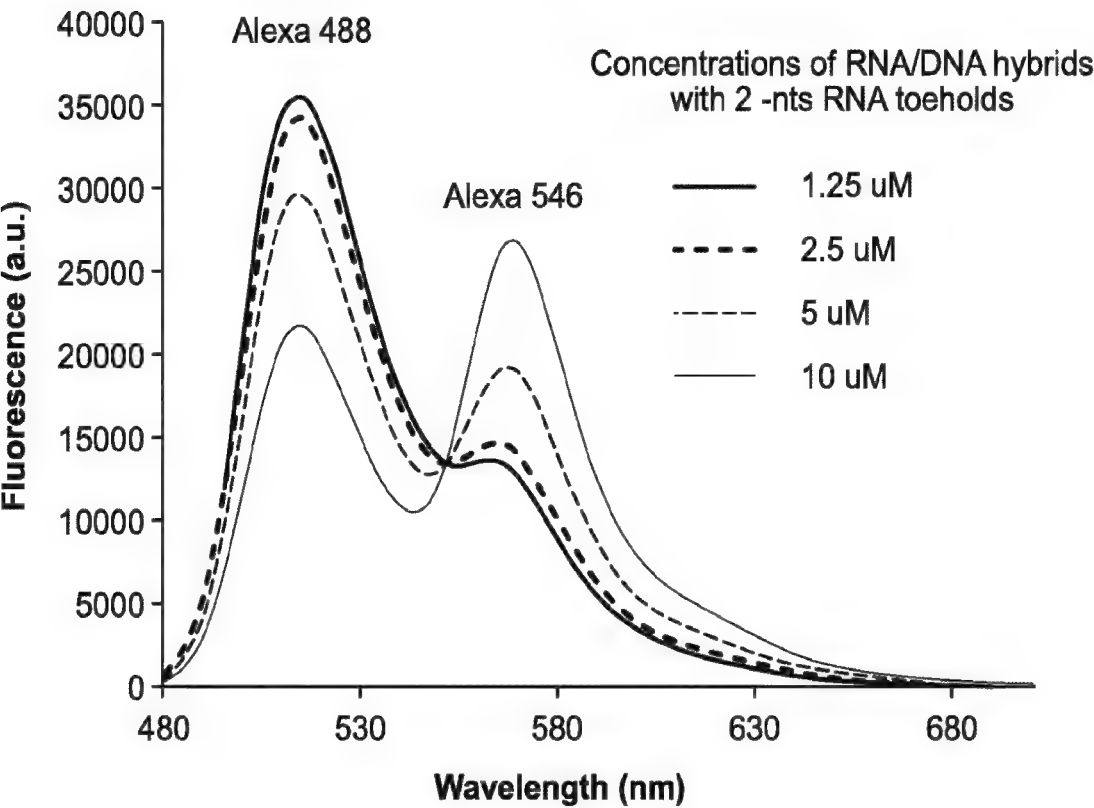
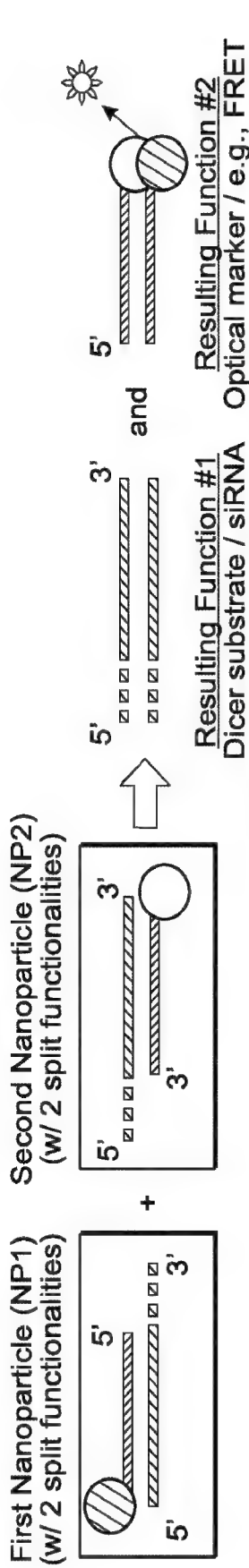


FIG. 1F

**Activatable Nanoparticle System**

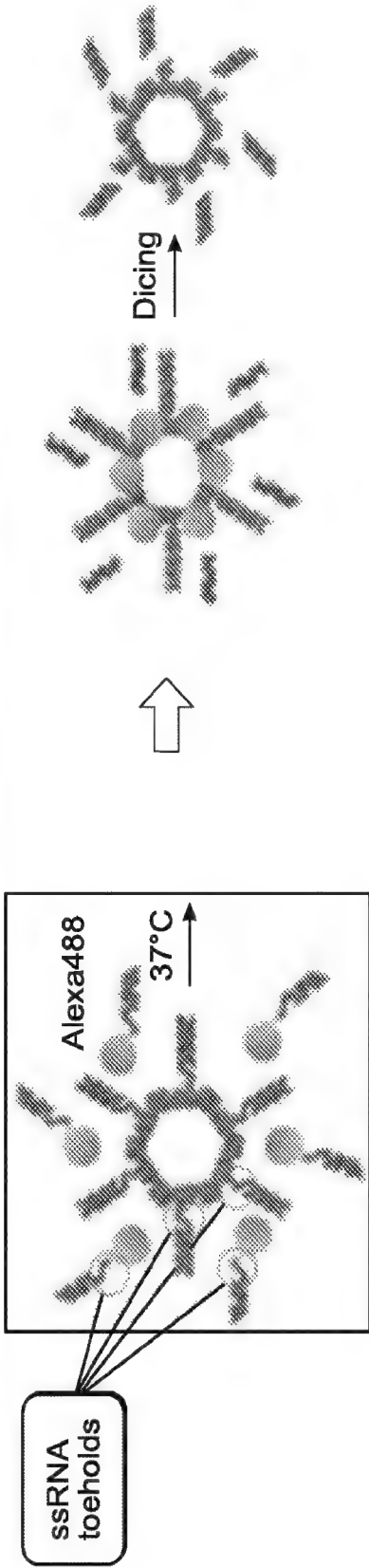
**FIG. 1G**

**Generalized schematic**



exemplary embodiments:  
NP1 = nanoring/nanocube with split-function hybrids with ssRNA toeholds  
NP2 = cognate split-function hybrids with ssRNA toeholds  
Split functionalities: (a) split siRNA or Dicer substrates, (b) split promoter elements for transcription, (c) split optical response elements (e.g., FRET), (d) split aptamer elements (e.g., Malachite green)

**NP1 = nanoring with split-function hybrids and NP2 = cognate hybrids**



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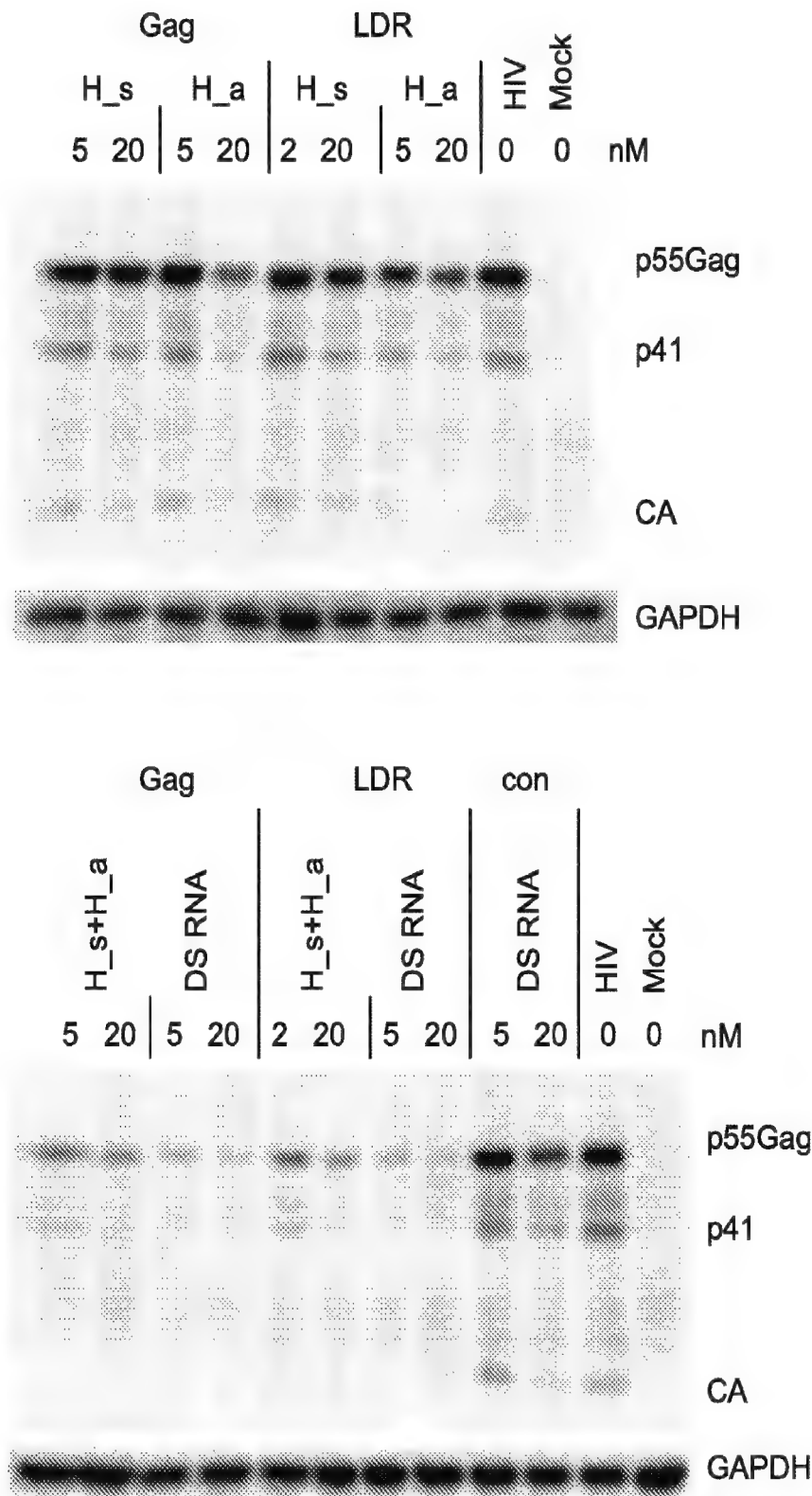


FIG. 2A

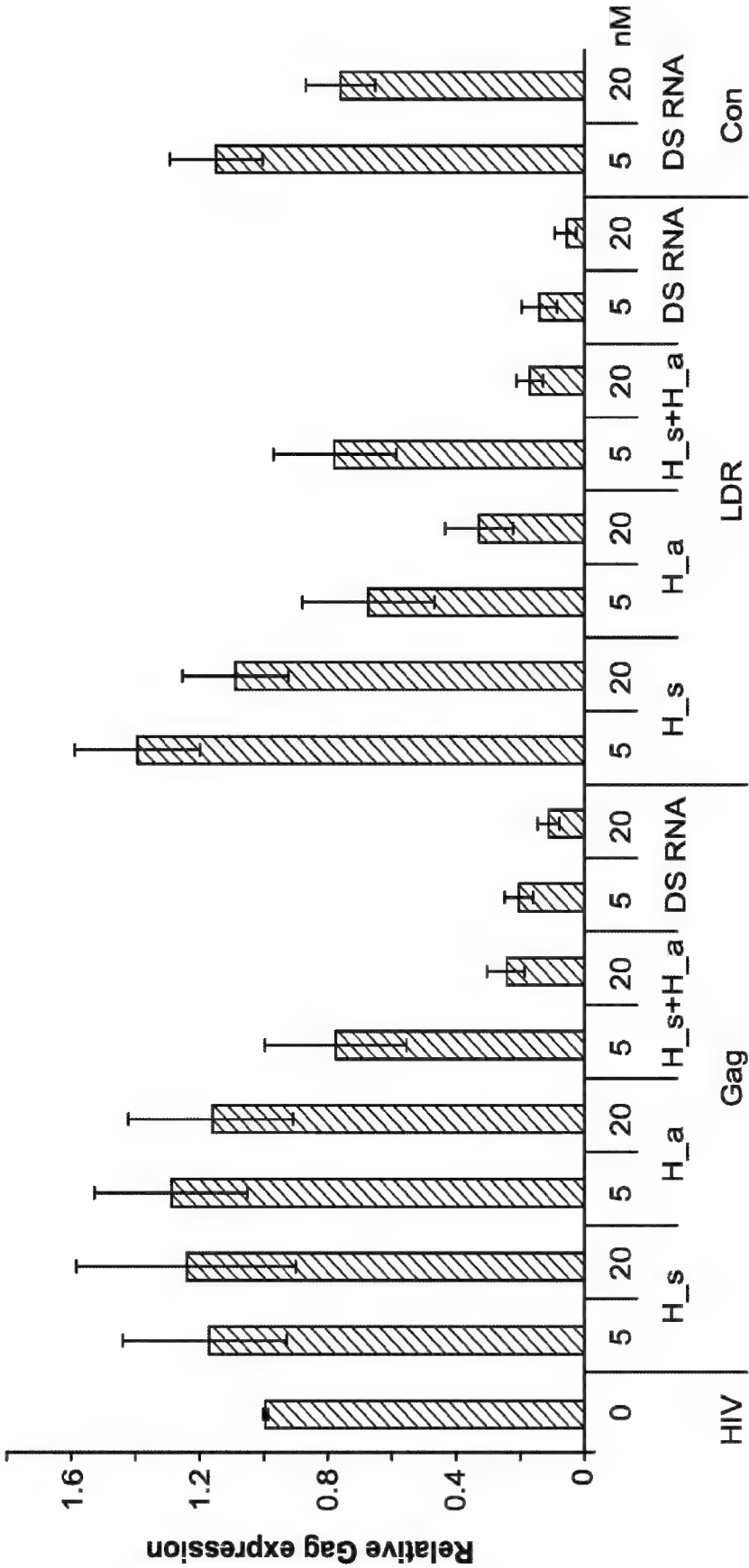


FIG. 2B

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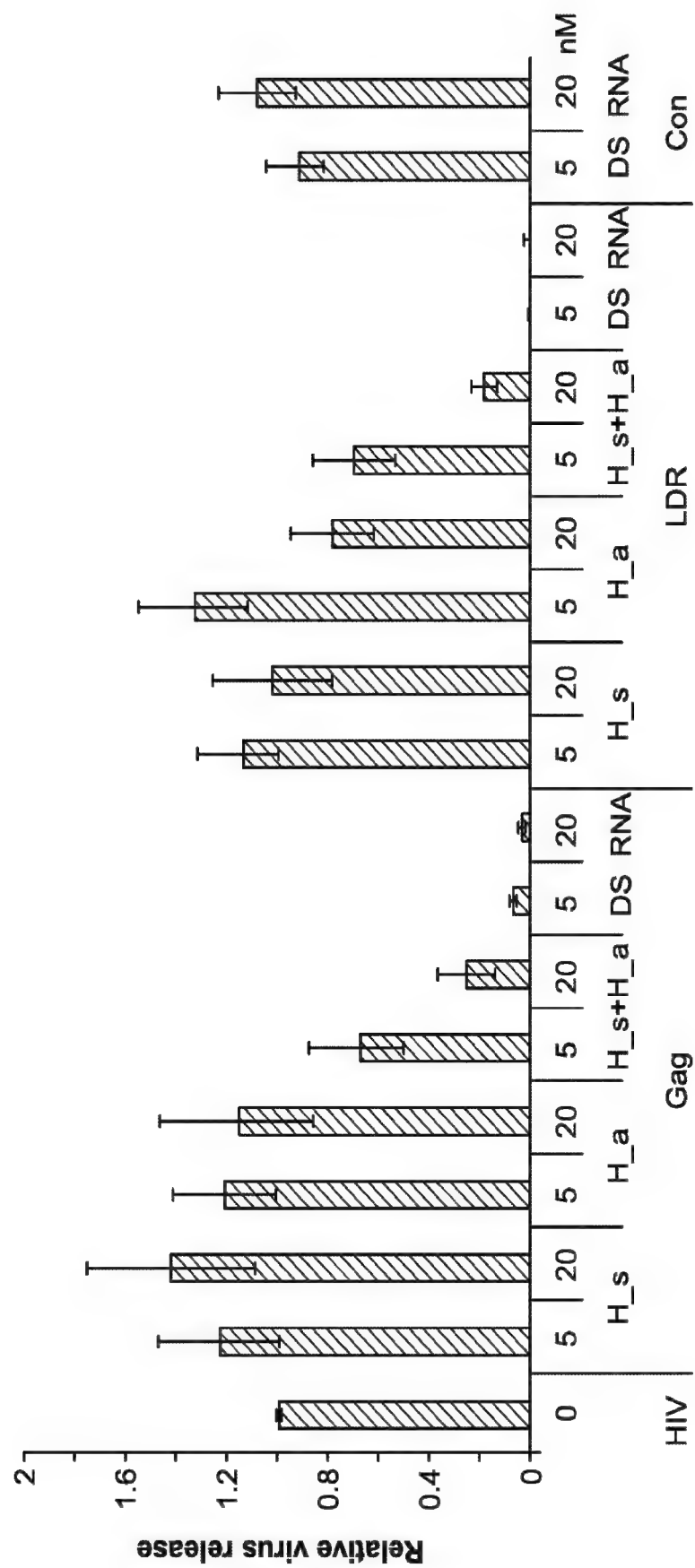


FIG. 2C



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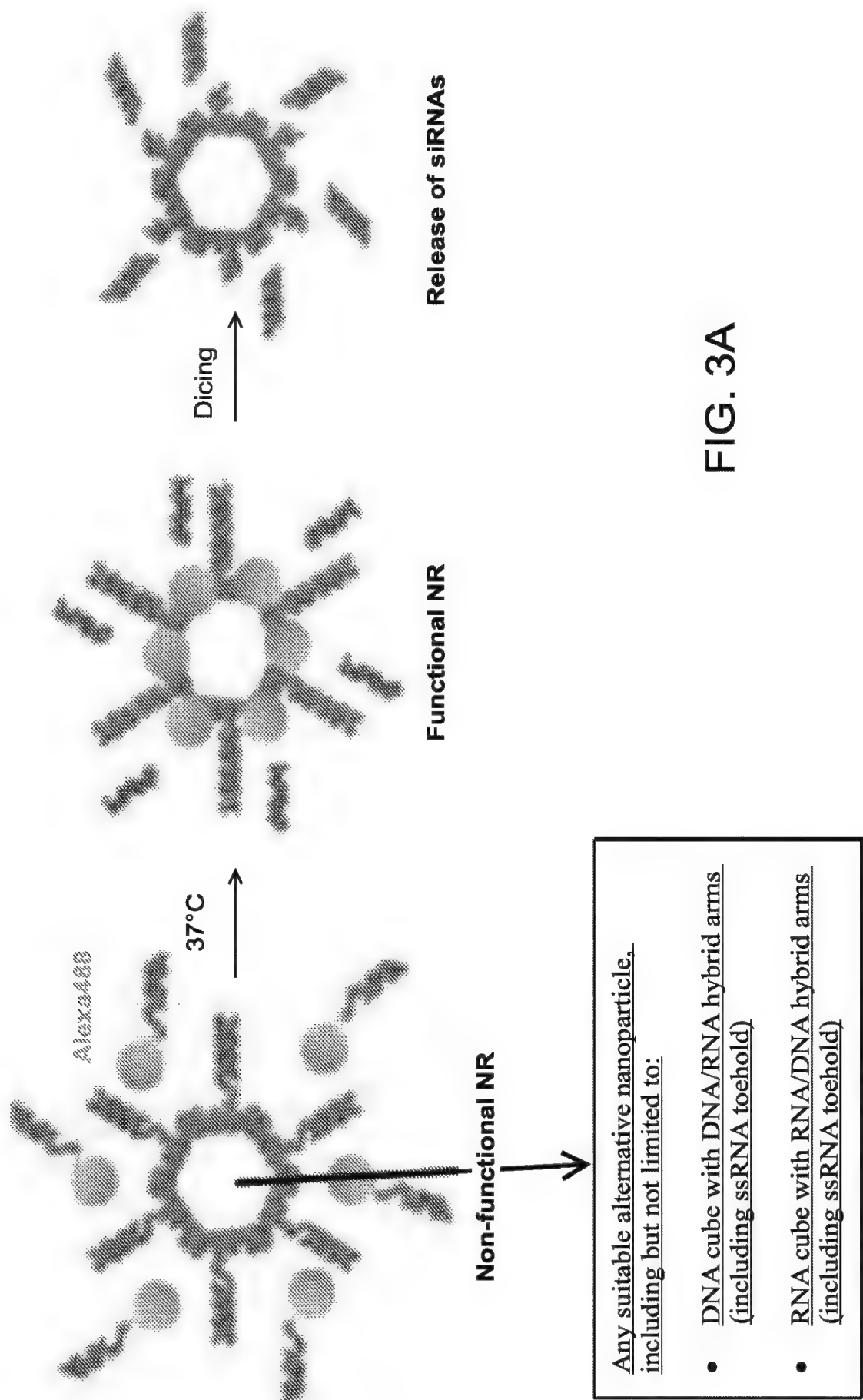


FIG. 3A

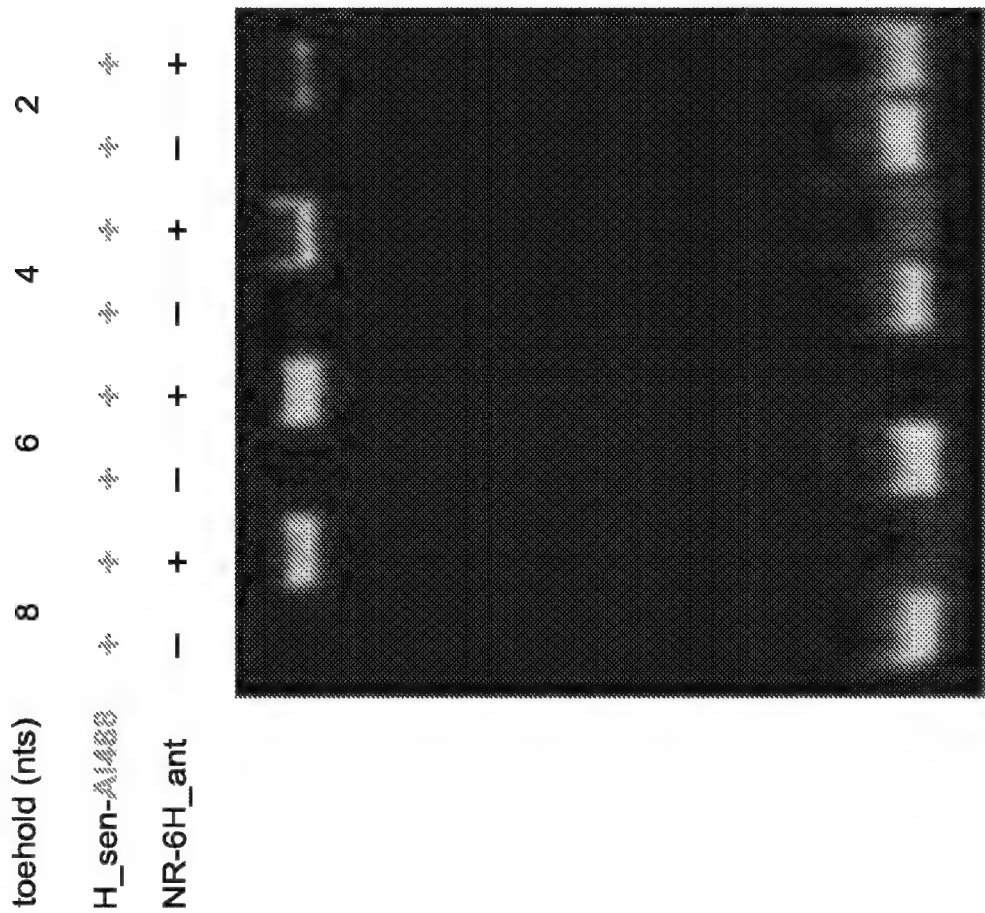
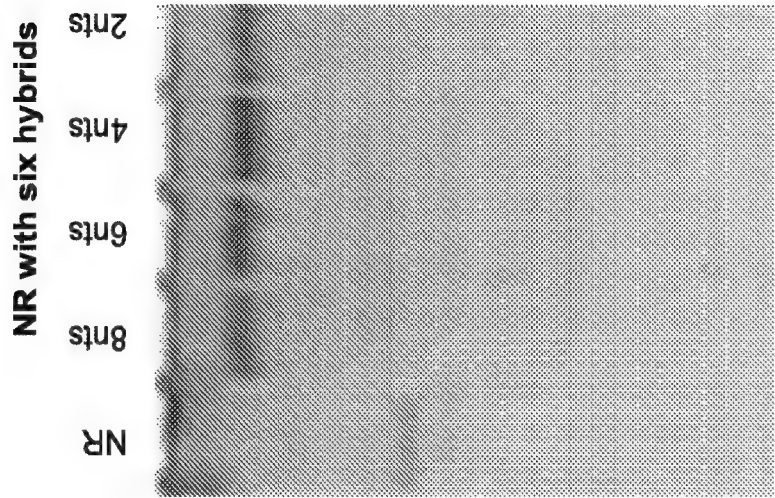


FIG. 3B





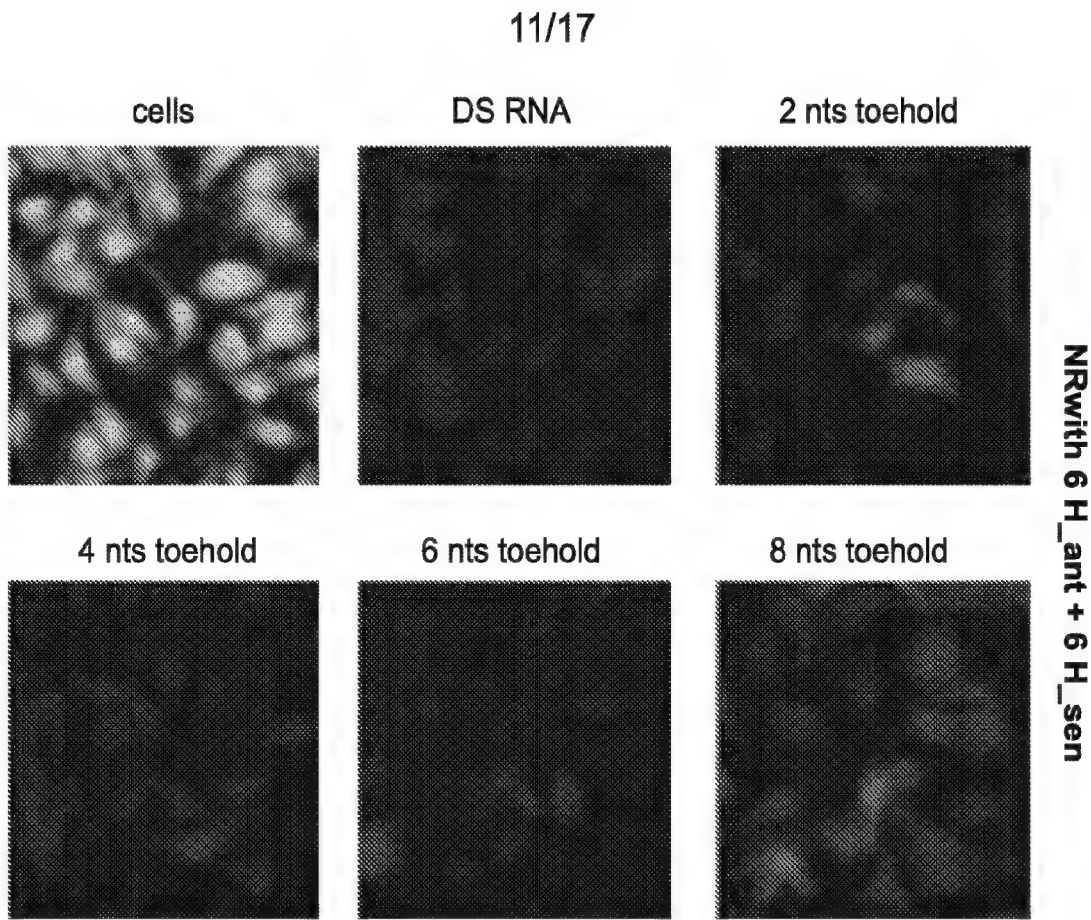


FIG. 3C

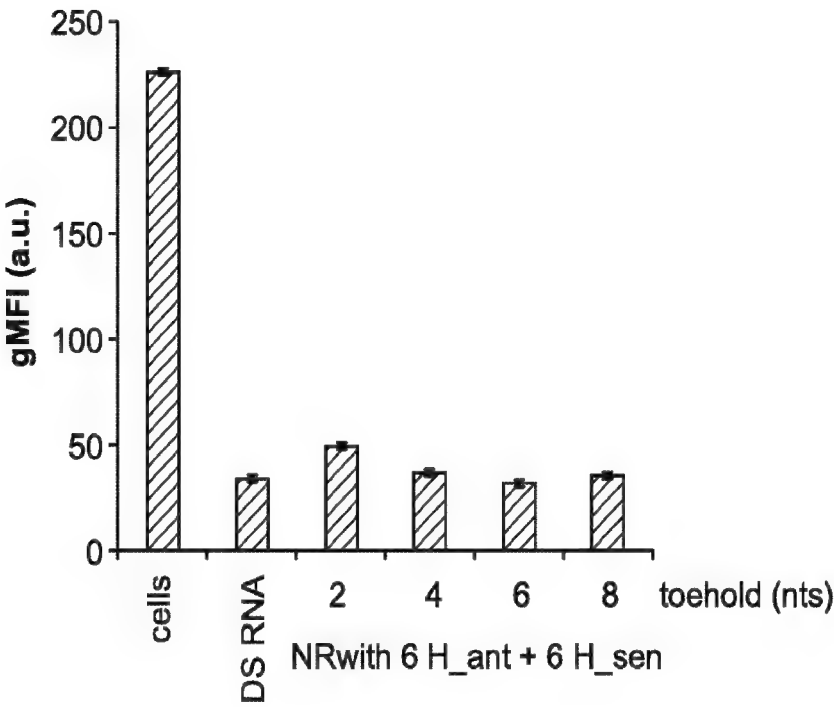


FIG. 3D

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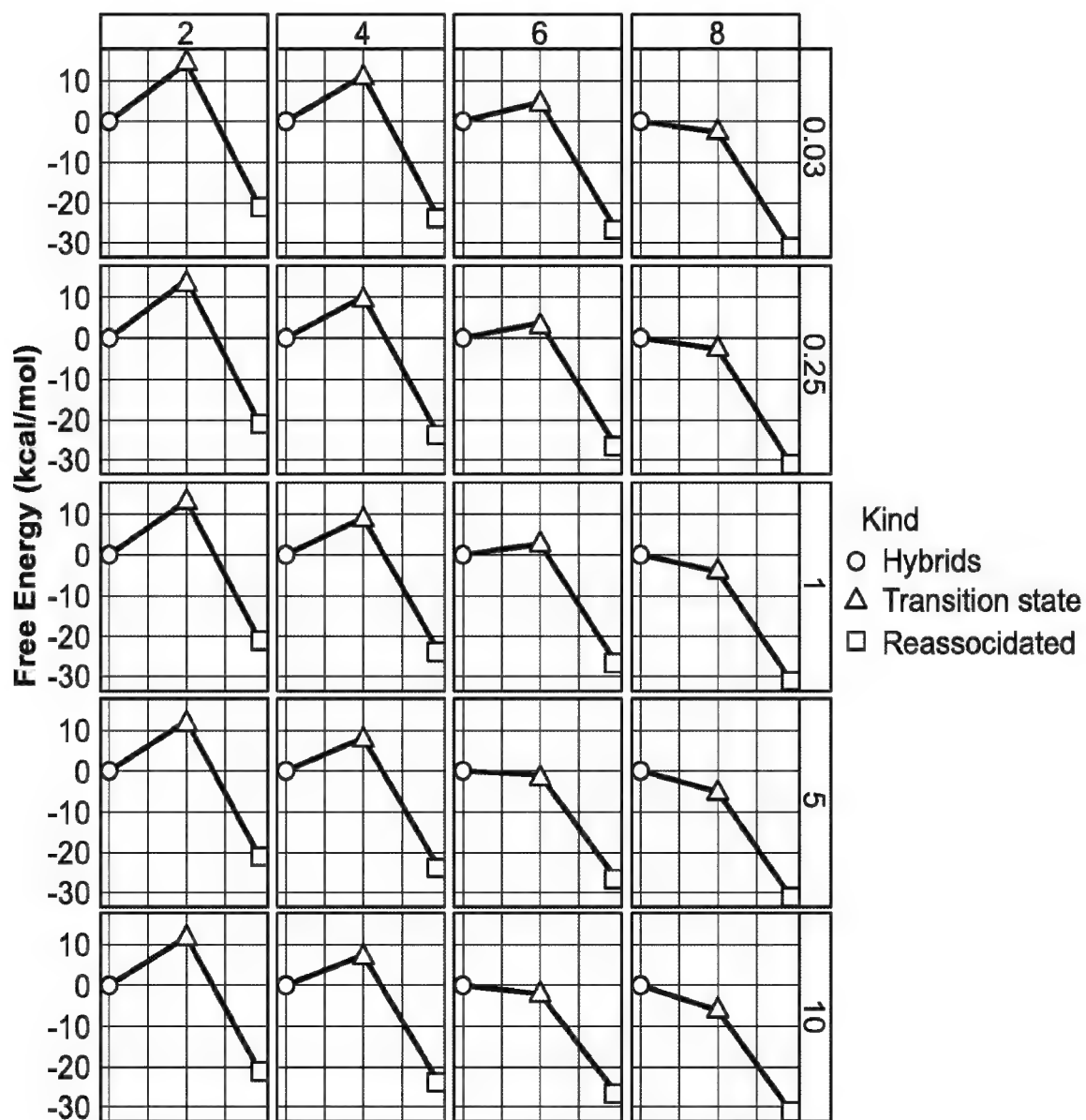


FIG. 4A

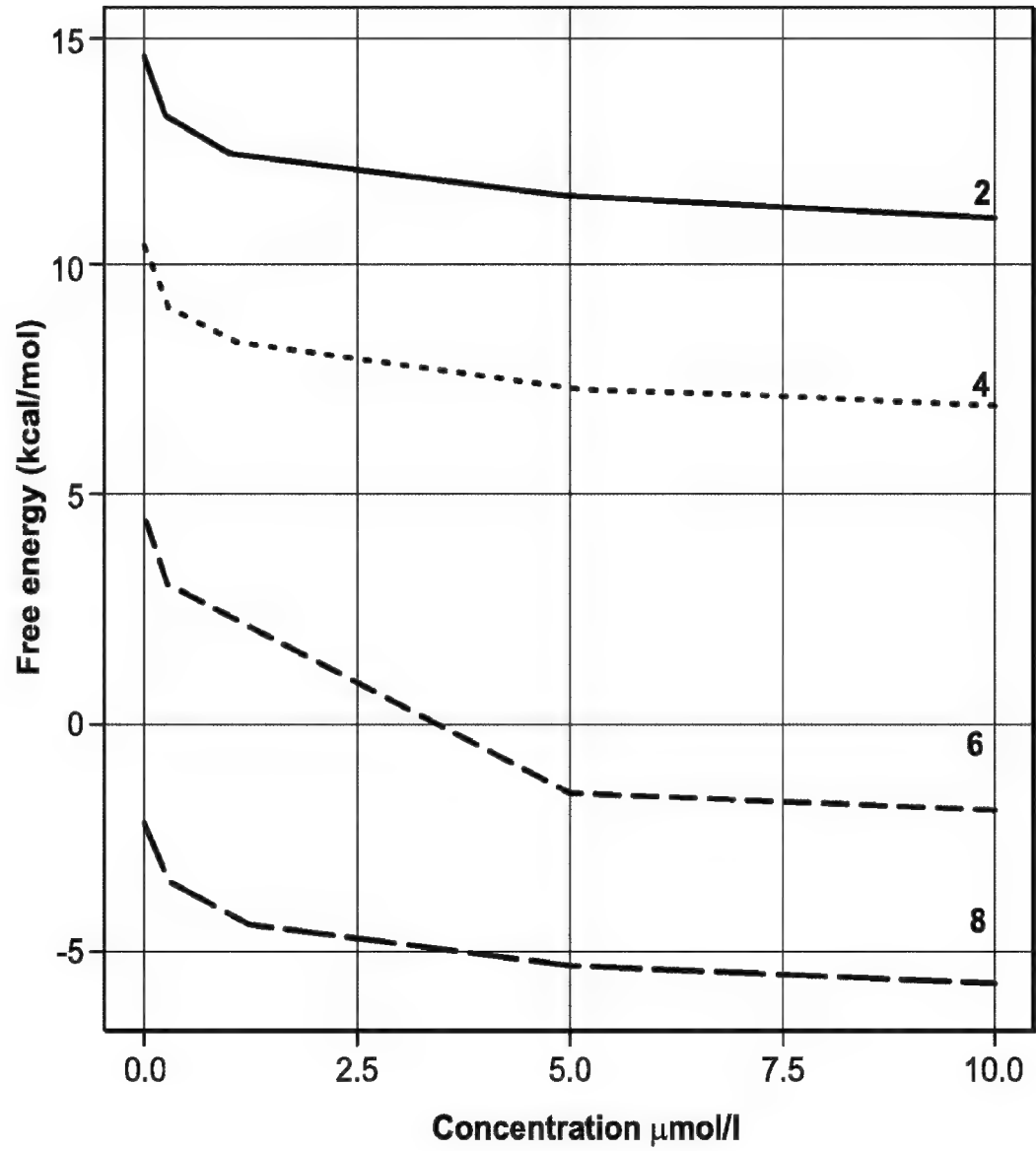


FIG. 4B

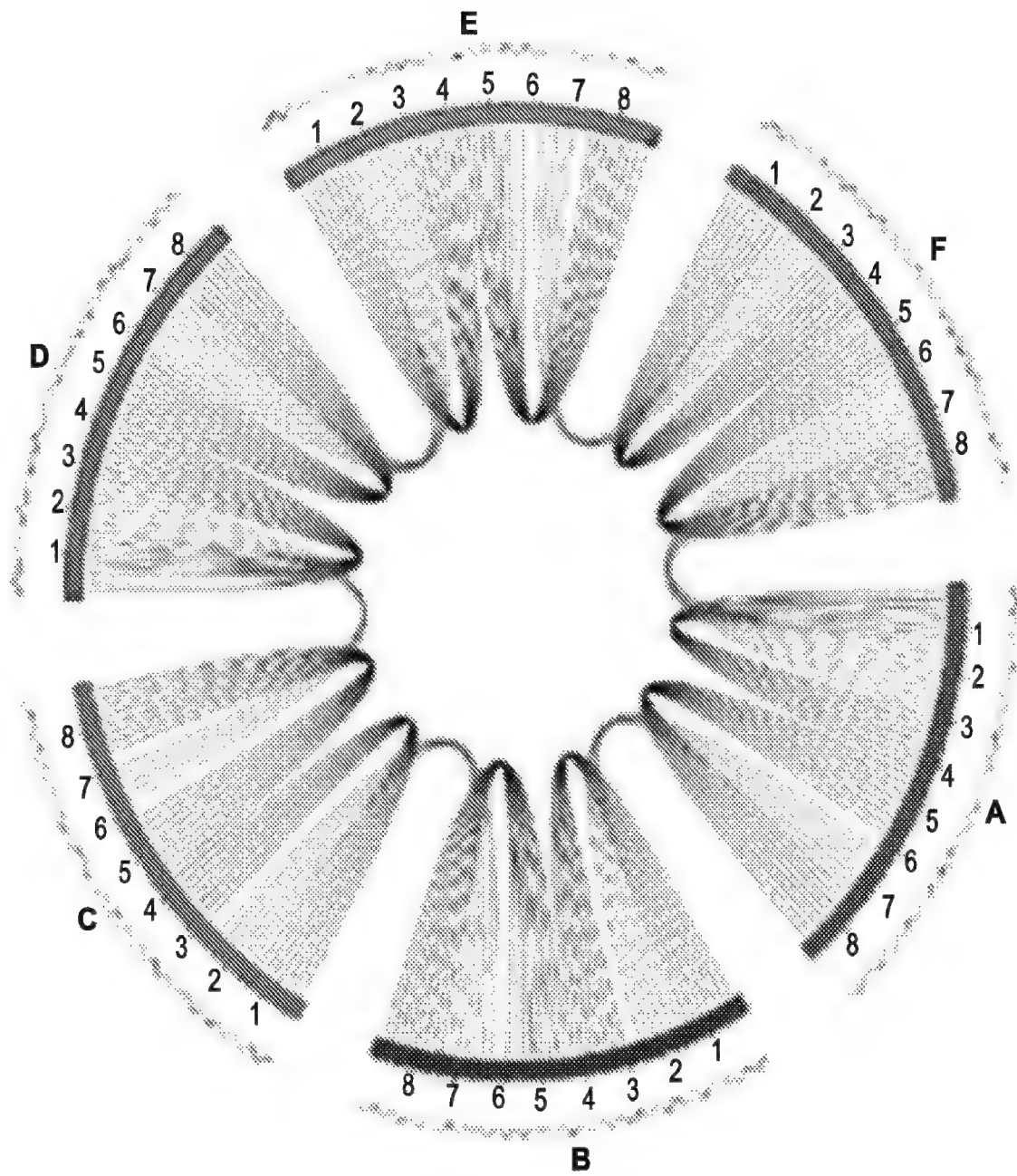


FIG. 4C



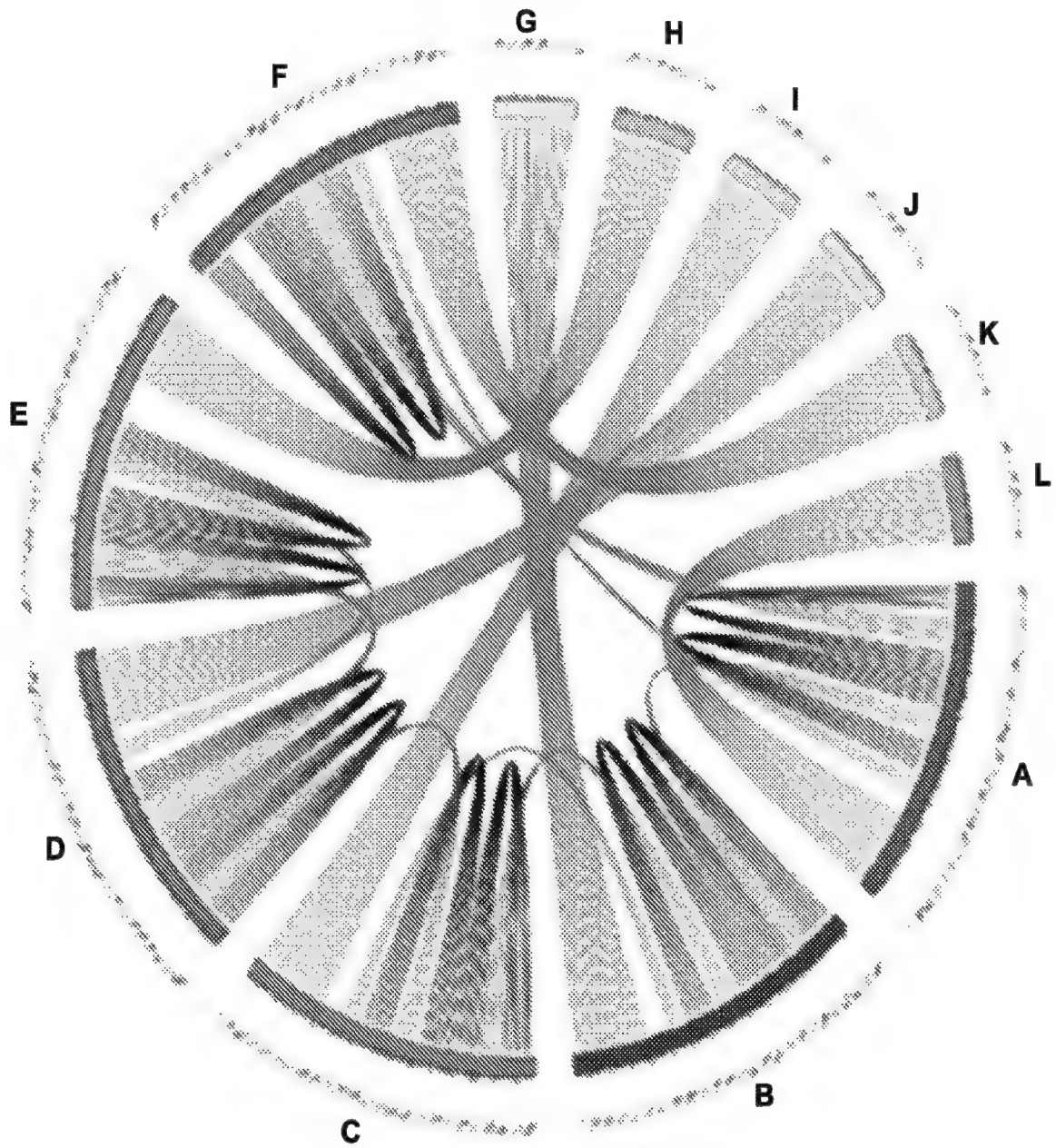


FIG. 4D



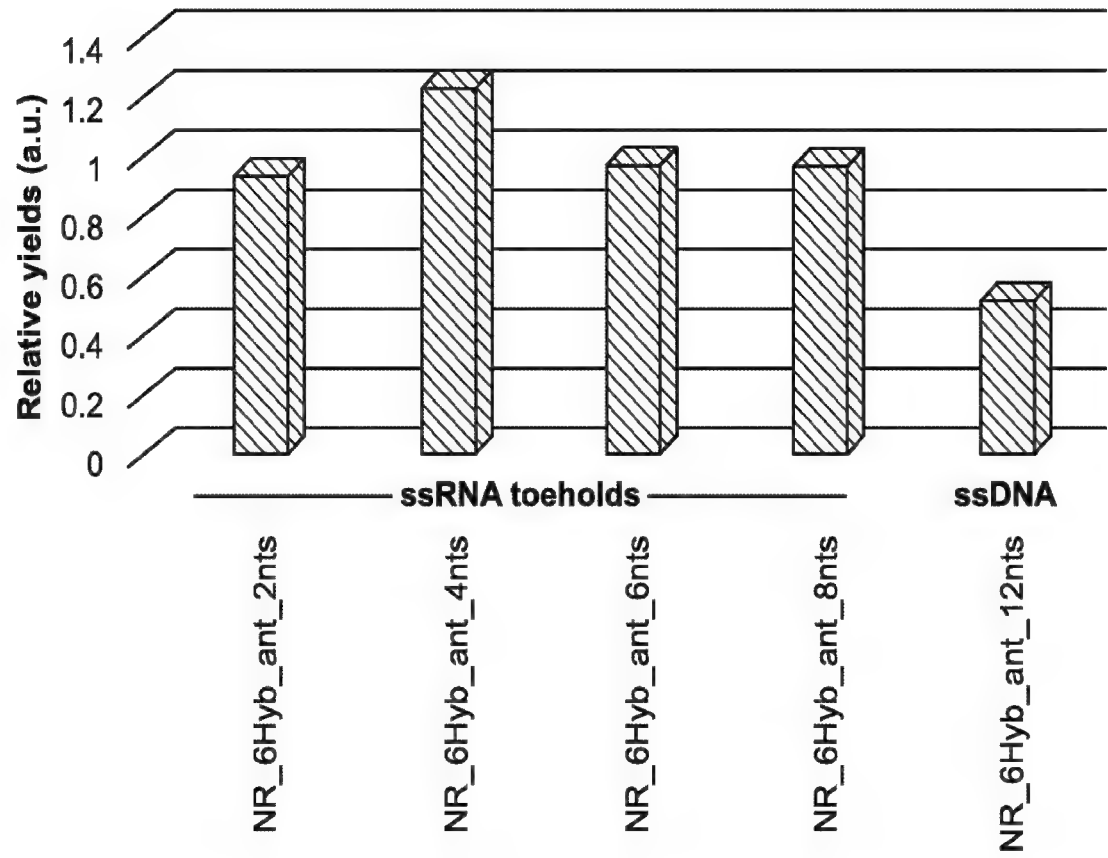


FIG. 5

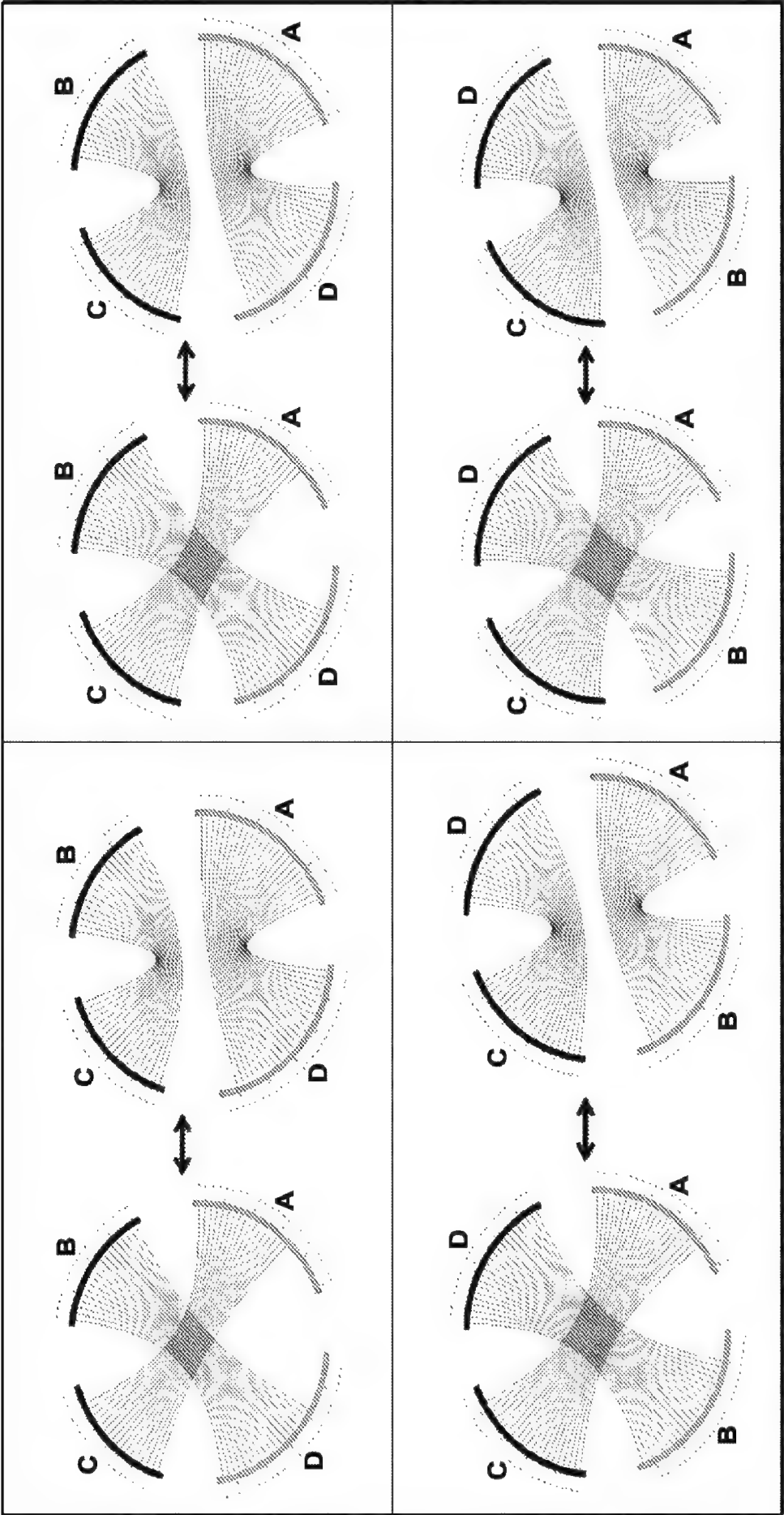


FIG. 6

## INTERNATIONAL SEARCH REPORT

International application No

PCT/US2017/017661

## A. CLASSIFICATION OF SUBJECT MATTER

INV. C12N15/113 B82Y5/00 A61K47/55  
 ADD. C12N15/87

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C12N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, BIOSIS, EMBASE, WPI Data

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2015/171827 A1 (US HEALTH [US]) 12 November 2015 (2015-11-12) cited in the application the whole document	1-25
Y	----- KIRILL A. AFONIN ET AL: "Multifunctional RNA Nanoparticles", NANO LETTERS, vol. 14, no. 10, 30 September 2014 (2014-09-30), pages 5662-5671, XP055164732, ISSN: 1530-6984, DOI: 10.1021/nl502385k cited in the application the whole document ----- -/--	1-25



Further documents are listed in the continuation of Box C.



See patent family annex.

## \* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&amp;" document member of the same patent family

Date of the actual completion of the international search

2 June 2017

Date of mailing of the international search report

13/06/2017

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2  
 NL - 2280 HV Rijswijk  
 Tel. (+31-70) 340-2040,  
 Fax: (+31-70) 340-3016

Authorized officer

Andres, Serge

## INTERNATIONAL SEARCH REPORT

International application No

PCT/US2017/017661

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2013/075132 A1 (US HEALTH [US]; UNIV CALIFORNIA [US]) 23 May 2013 (2013-05-23) cited in the application the whole document	1-25
A	----- Kirill A. Afonin ET AL: "Computational and Experimental Studies of Reassociating RNA/DNA Hybrids Containing Split Functionalities" In: "METHODS IN ENZYMOLOGY", 2015, ACADEMIC PRESS, US, XP055377414, ISSN: 0076-6879 vol. 553, pages 313-334, DOI: 10.1016/bs.mie.2014.10.058, the whole document	1-25
A	----- K. A. AFONIN ET AL: "Co-transcriptional production of RNA-DNA hybrids for simultaneous release of multiple split functionalities", NUCLEIC ACIDS RESEARCH, vol. 42, no. 3, 4 November 2013 (2013-11-04), pages 2085-2097, XP055164770, ISSN: 0305-1048, DOI: 10.1093/nar/gkt1001 cited in the application the whole document	1-25
A	----- KIRILL A. AFONIN ET AL: "Triggering of RNA Interference with RNA-RNA, RNA-DNA, and DNA-RNA Nanoparticles", ACS NANO, 18 December 2014 (2014-12-18), XP055164747, ISSN: 1936-0851, DOI: 10.1021/nn504508s cited in the application the whole document	1-25
A,P	----- LORENA PARLEA ET AL: "Cellular Delivery of RNA Nanoparticles", ACS COMBINATORIAL SCIENCE, vol. 18, no. 9, 12 September 2016 (2016-09-12), pages 527-547, XP055377364, US ISSN: 2156-8952, DOI: 10.1021/acscmbosci.6b00073 cited in the application the whole document ----- -/--	1-25

# INTERNATIONAL SEARCH REPORT

International application No

PCT/US2017/017661

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	<p>KIRILL A. AFONIN ET AL: "The Use of Minimal RNA Toeholds to Trigger the Activation of Multiple Functionalities", NANO LETTERS, vol. 16, no. 3, 9 March 2016 (2016-03-09), pages 1746-1753, XP055377367, US</p> <p>ISSN: 1530-6984, DOI: 10.1021/acs.nanolett.5b04676</p> <p>cited in the application</p> <p>the whole document</p> <p>-----</p>	1-25

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2017/017661

### Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
- a. ☐ forming part of the international application as filed:
- ☐ in the form of an Annex C/ST.25 text file.
- ☐ on paper or in the form of an image file.
- b. ☐ furnished together with the international application under PCT Rule 13~~ter~~.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
- c. ☒ furnished subsequent to the international filing date for the purposes of international search only:
- ☒ in the form of an Annex C/ST.25 text file (Rule 13~~ter~~.1(a)).
- ☐ on paper or in the form of an image file (Rule 13~~ter~~.1(b) and Administrative Instructions, Section 713).
2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:



# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2017/017661

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2015171827 A1	12-11-2015	NONE	
WO 2013075132 A1	23-05-2013	AU 2012340086 A1	29-05-2014
		CA 2856116 A1	23-05-2013
		EP 2780456 A1	24-09-2014
		JP 2014533953 A	18-12-2014
		US 2015240238 A1	27-08-2015
		WO 2013075132 A1	23-05-2013



**b5**

---

**From:** jamespackardlove@gmail.com [jamespackardlove@gmail.com]  
**on behalf of** Jamie Love [james.love@keionline.org]  
**Sent:** 1/12/2017 11:17:58 PM  
**To:** Rohrbaugh, Mark (NIH/OD) [E] [/O=NIH/OU=NIHEXCHANGE/cn=OD/cn=ROHRBAUM]  
**Subject:** Re: Thanks for today

Going forward, we will be, not surprisingly, trying to get more transparency of the CRADA and licensing practices. At some point, having a conversation about how best this can be done would be good, if the NIH itself saw greater transparency is a legitimate and useful obligation on the agency.

Like many others, we are supportive of endless increases in the NIH budget, and supportive of its mission. We are sort of surprised it is so difficult to get information about the licenses and CRADAs.

Jamie

On Thu, Jan 12, 2017 at 6:10 PM, Rohrbaugh, Mark (NIH/OD) [E] <[RohrBauM@od.nih.gov](mailto:RohrBauM@od.nih.gov)> wrote:

My pleasure

**From:** [jamespackardlove@gmail.com](mailto:jamespackardlove@gmail.com) [mailto:[jamespackardlove@gmail.com](mailto:jamespackardlove@gmail.com)] **On Behalf Of** Jamie Love  
**Sent:** Thursday, January 12, 2017 6:07 PM  
**To:** Rohrbaugh, Mark (NIH/OD) [E] <[RohrBauM@OD.NIH.GOV](mailto:RohrBauM@OD.NIH.GOV)>  
**Subject:** Thanks for today

Claire learned a lot. We clearly did not understand the CRADA process that well. And thanks for explaining things.

Jamie

--

James Love. Knowledge Ecology International  
<http://www.keionline.org/donate.html>

KEI DC tel: [+1.202.332.2670](tel:+12023322670), US Mobile: [+1.202.361.3040](tel:+12023613040), Geneva Mobile: [+41.76.413.6584](tel:+41764136584),  
[twitter.com/jamie\\_love](https://twitter.com/jamie_love)

--

James Love. Knowledge Ecology International

<http://www.keionline.org/donate.html>

KEI DC tel: +1.202.332.2670, US Mobile: +1.202.361.3040, Geneva Mobile: +41.76.413.6584,

[twitter.com/jamie\\_love](https://twitter.com/jamie_love)

---

**From:** Fine, Amanda (NIH/OD) [E] [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=61290B74AA9A44358954C45439FFDEB6-FINEAB]  
**Sent:** 4/16/2019 9:12:01 PM  
**To:** Rohrbaugh, Mark (NIH/OD) [E] [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=591ab6b2424b4b8997082718cbb29fab-rohrbaum]; Wojtowicz, Emma (NIH/OD) [E] [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=45c6610aca6e44a08d497630425e5ecd-wojtowiczem]  
**Subject:** RE: Background info on CAR T license to Gilead  
**Attachments:** Interview request: exclusive license for a CAR-T therapy

Hi Mark-

Sorry for the delay--are you looking for the attached?

Thanks,  
Amanda

-----Original Message-----

**From:** Rohrbaugh, Mark (NIH/OD) [E] <rohrbaum@od.nih.gov>  
**Sent:** Tuesday, April 16, 2019 12:06 PM  
**To:** Fine, Amanda (NIH/OD) [E] <amanda.fine@nih.gov>; Wojtowicz, Emma (NIH/OD) [E] <emma.wojtowicz@nih.gov>  
**Subject:** FW: Background info on CAR T license to Gilead

Do you have the sheet we prepared for reporters on the CAR-T technology last year?

Thanks,

-----Original Message-----

**From:** Joe Allen <jallen@allen-assoc.com>  
**Sent:** Tuesday, April 16, 2019 11:01 AM  
**To:** Rohrbaugh, Mark (NIH/OD) [E] <rohrbaum@od.nih.gov>  
**Subject:** Background info on CAR T license to Gilead

**b4**

Thanks

--

Joseph P. Allen  
President  
Allen and Associates  
60704 Rt. 26, South  
Bethesda, OH 43719  
(W) 740-484-1814  
(C) b6  
www.allen-assoc.com

REL0000024033

---

**From:** Fine, Amanda (NIH/OD) [E] [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=61290B74AA9A44358954C45439FFDEB6-FINEAB]  
**Sent:** 12/6/2018 7:56:24 PM  
**To:** HHS/OS Interviews [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=0e101dc31e9c4c8689b4bd64dddc01f4-interviews.]  
**CC:** Burklow, John (NIH/OD) [E] [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=2e57f267323b43c08be856acb5b964ca-burklowj]; Prince, Scott (NIH/OD) [E] [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=0fd557b0e1194e36bc407110cb681025-princes]; OCPLPressTeam [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=53ba510dfce44512b4a76db41c90df84-OCPL Press]; ODOCPL Interviews (NIH/OD OCPL ) [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=169257452ee5414f84624bd1d29c3f5c-ODOCPL Inte]  
**Subject:** Interview request: exclusive license for a CAR-T therapy

Reporter: Ed Silverman  
Organization: STAT  
Phone #(s): ed.silverman@statnews.com  
Subject: exclusive license for a CAR-T therapy  
Deadline: today  
Spokesperson: NIH general  
Expected place of publication (print, online, broadcast): online  
Expected date of publication/airing: tbd  
Expected prominence (e.g. front page, Sunday, evening/morning show, etc.): tbd

Key messages/talking points:

Thank you for contacting the NIH with your questions and concerns. Many, if not most, of the technologies developed at the NIH are early stage biomedical technologies that require a great deal of further development and regulatory approval before they can be provided to patients. The time, cost and risks to develop an FDA-approved drug or vaccine are high (estimated to be at least \$1 billion per drug), and only about one-tenth of a percent (0.1%) of potential drug candidates are eventually approved by the FDA. NIH supports research to identify the mechanisms of disease and the discovery of early stage technologies (an area of research generally not supported by the private sector), but relies on the private sector to bring products through the full pre-clinical and clinical process, the most expensive stages of drug development. Through the NIH's licensing program, the private sector takes early stage technologies and develops them into new treatments to benefit public health. The NIH grants exclusive licenses to incentivize companies to invest in the development of early stage NIH technologies so they can benefit public health. Without such an incentive, these early stage technologies would likely never be developed.

When a company requests an exclusive license, NIH considers carefully whether the technology should be made available on an exclusive or non-exclusive basis for that purpose. NIH's interest in licensing its inventions is public and transparent. Beginning in 2016, NIH advertised on the NCI Tech Transfer and on NIH Technology Transfer websites that the NIH invention (chimeric antigen receptor (CAR) modified T cells to target FLT3) was being made available for licensing. The invention is an adoptive cellular therapy that can be licensed in multiple different iterations based on the method of CAR delivery (viral vectors, genome editing), modality of treatment (allogeneic vs autologous cell therapy), and as one CAR in a multi-CAR approach to treat cancer. FLT3 is a protein found on blood cells that is often elevated in some leukemias, and scFvs are the portion of the CAR construct that binds to a particular region of a specific protein, thus allowing for antigen specificity of the modified T cell. For this license, the proposed scope will be no broader than a lentivirus delivery system, one of various types of viruses that can be used, and the scFv is limited to one referenced as NC7. NIH owns rights to additional FLT3-targeting CARs that are available for licensing based on alternative scFvs. There are, therefore, a large number of licensable products available from the NIH as adoptive cell therapies for FLT3 cancers, all of which were advertised. The NIH advertisements remain active, and NIH

continues to seek commercialization partners for unlicensed uses of the invention. Universities and the private sector have developed CAR technologies that may be alternatives to the NIH technologies.

Prior to posting a public notice in the federal register for a proposed granting of an exclusive license, the NIH determines that the legal criteria set forth in 37 CFR 404.7(a)(1)(ii-iii) have been satisfied and that a company is qualified to be granted an exclusive license to the Government's intellectual property in the fields of use as specified.

ElevateBio applied for an exclusive license for the development of one of these classes of FLT3-targeting CARs, which NIH is currently considering. As of the date of posting in the federal register, ElevateBio is the only company that has applied for a license for this particular aspect of the invention. ElevateBio has demonstrated that their researchers have significant expertise with the aspect of the inventions that they would like to license, and NIH has determined that ElevateBio is a strong licensing candidate.

Licenses for developing the other classes of FLT3 targeting CARs are still available for other companies to pursue.

By taking a licensing approach that separates out the invention into classes, and by then licensing those classes separately to different companies, the NIH fosters competition in the drug market, which may lead to competitive drug pricing.

We hope that the explanation above provides some assurance that any decision NIH makes to license one of its technologies involves a lot of thought and diligence as well as compliance with federal laws and regulations.

Additional information: Ed regularly covers letters KEI puts out. He saw this one: [https://www.keionline.org/wp-content/uploads/2018/12/83FR58262\\_Comments\\_NIH\\_ElevateBIO\\_4Nov2018.pdf](https://www.keionline.org/wp-content/uploads/2018/12/83FR58262_Comments_NIH_ElevateBIO_4Nov2018.pdf) and asked why NIH chose this company to grant an exclusive license. This is our standard response with details of this specific technology and license.

**From:** Shmilovich, Michael (NIH/NHLBI) [E] [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=7DFE19BFD1D443CEB700B9F22D159A90-SHMILOVM]  
**Sent:** 8/20/2018 2:56:53 PM  
**To:** Rohrbaugh, Mark (NIH/OD) [E] [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=591ab6b2424b4b8997082718cbb29fab-rohrbaum]; Deutch, Alan (NIH/NHLBI) [E] [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=244d755700584812af36b5e787285647-deutch]  
**CC:** 'Francois Ravenelle' [fravenelle@inversago.com]  
**Subject:** KEI -T1International-NIH-InversagoPharma-20aug2018.pdf  
**Attachments:** KEI -T1International-NIH-InversagoPharma-20aug2018.pdf

Dear Mark –

b5

b5

Regards,

**Michael A. Shmilovich, Esq., CLP**



National Heart, Lung,  
and Blood Institute

Office of Technology Transfer and Development  
31 Center Drive Room 4A29, MSC2479  
Bethesda, MD 20892-2479  
o. 301.435.5019  
[shmilovm@nih.gov](mailto:shmilovm@nih.gov)

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REL0000024034



August 20, 2018

Michael Shmilovich, Esq.,  
Senior Licensing and Patent Manager,  
31 Center Drive Room 4A29, MSC2479,  
Bethesda, MD 20892-2479,  
Email: [shmilovm@mail.nih.gov](mailto:shmilovm@mail.nih.gov).

RE: Prospective Grant of Exclusive Patent License: Treatment of Type 1 Diabetes and its Comorbidities, 83 FR 38707 (<https://www.federalregister.gov/d/2018-16836>), to Inversago Pharma, a firm headquartered in Canada.

Dear Michael Shmilovich, Esq.:

Knowledge Ecology International (KEI) and T1International jointly provide the following comments on the prospective grant of an exclusive patent license for human therapeutics for type 1 diabetes to a Montreal-based company called Inversago Pharma, Inc., as noticed on August 7, 2018 in the Federal Register (83 FR 38707).

KEI is a non-profit organization with offices in Washington, DC and Geneva, Switzerland, that focuses on the management of knowledge goods, including medical inventions. KEI is focused on improving innovation for and access to new medical technologies. Our comments to the NIH in this and in several other matters often focus on the responsibility of the NIH to ensure that subject inventions are available to the public on reasonable terms, and that exclusive rights are limited to those that are reasonably necessary to induce the investments needed to bring the invention to practical application. KEI is concerned about the impact of high prices on the affordability of and access to medical treatments, both in the United States and for vulnerable populations in developing countries.

T1International is a non-profit run by people with and impacted by type 1 diabetes, for people with type 1 diabetes. T1International is active around the world in efforts to fight against high prices for insulin and other treatments essential for persons living with type 1 diabetes.

According to the notice published in the Federal Register, the prospective exclusive patent license territory, "will be granted worldwide and in a field of use not broader than human therapeutics for type I diabetes and its comorbidities diabetic nephropathy, chronic kidney disease, diabetic retinopathy, and peripheral and autonomic neuropathy."

Inversago Pharma, Inc. is a company located in Canada, that is "focused on the development of peripherally-restricted CB1 receptor (CB1) inverse agonists for the treatment of Prader-Willi Syndrome, type 1 diabetes and metabolic disorders in general," according to the description

provided on the Inversago website.<sup>1</sup> This company has several investigational drugs in their pipeline targeting four indications, but only one of those investigational drugs (for Prader-Willi Syndrome) has reached preclinical stage.<sup>2</sup>

Based on information provided in the Inversago website<sup>3</sup> and the Federal Register notice 80 FR 66015 from October 28, 2015<sup>4</sup>, Inversago already holds a license (which was executed<sup>5</sup>) to several NIH-owned inventions related to “CB1 receptor mediating compounds.”

NIH scientist George Kunos appears as an inventor in the published applications or issued patents listed in that October 28, 2015 Federal Register notice, as well as in the published or issued patents cited in the 2018 notice.

The geographical scope for the license in the 2015 notice was “worldwide” and the field of use was for the development of therapeutics for “obesity, type 2 diabetes, fatty liver disease and liver fibrosis in humans.”<sup>6</sup>

The new exclusive license the NIH proposes for Inversago does two things. First, the NIH will effectively expand the field of use for all of the patents noticed by the NIH in 2015, so that the inventions can be used to treat type 1 diabetes. Second, the NIH includes several new granted patents or patent applications, which will also be licensed to treat type 1 diabetes.

In July 25, 2018, Inversago Pharma Inc announced that it had secured \$7 million in financing “to pursue its development plan and advance its CB1 platform as a potent treatment for targeted diseases,” including Prader-Willi Syndrome (PWS) and type 1 diabetes.<sup>7</sup> Development of human therapeutics for type 1 diabetes based on CB1 is presumably outside of the field of use of the exclusive NIH license Inversago currently holds.

Inversago notes on its website that the technology for which it had secured \$7 million in financing was “based on the work by CB1 world expert George Kunos” at the NIH.<sup>8</sup>

The Federal Register notice 83 FR 38707 published on August 7, 2018 makes no reference to the previous exclusive license agreement executed between Inversago and the NIH. To the general public, the connection between the license agreement noticed in 2015 and executed in

---

<sup>1</sup> <https://web.archive.org/web/20180817175917/https://inversago.com/en/approach/>

<sup>2</sup> <https://web.archive.org/web/20180727110313/https://inversago.com/en/our-pipeline/>

<sup>3</sup> <https://web.archive.org/web/20180817175003/https://inversago.com/en/news-and-events/>

<sup>4</sup> <https://www.federalregister.gov/d/2015-27454>

<sup>5</sup> The exclusive license agreement between Inversago Pharma Inc and the NIH, described in the notice 80 FR 66015, was executed on April 26, 2016, according to the company's website:

<https://web.archive.org/web/20180817175003/https://inversago.com/en/news-and-events/>

<sup>6</sup> <https://www.federalregister.gov/d/2015-27454>

<sup>7</sup> <https://web.archive.org/web/20180817175003/https://inversago.com/en/news-and-events/>

<sup>8</sup> <https://web.archive.org/web/20180817175003/https://inversago.com/en/news-and-events/>

2016 and the prospective exclusive license noticed in 83 FR 38707 should have been made more clear, in the new notice published by the NIH.

Aside from the press release by Inversago stating that the company had secured \$7 million in financing, there are no publicly available reports of the efforts or investments done by Inversago to bring into practice the inventions covered in the first license agreement. The NIH has not provided an explanation of whether and how the prospective exclusive license noticed in 83 FR 38707 will expand the existing exclusive license between the NIH and Inversago; nor has it provided an explanation of how the NIH has determined that expanding the exclusive license granted to this company is a reasonable and adequate incentive to induce development, given the existing obligation to bring the inventions to practical application for type 2 diabetes.

KEI and T1International ask the NIH if it is satisfied that Inversago is, in fact, on track to bring the inventions licensed in 2016 to practical application? And if so, what is to be gained by granting an exclusive license to Inversago for use to treat type 1 diabetes? Until the NIH explains this, we oppose creating a monopoly on the NIH-owned inventions for type 1 diabetes.

Here are some additional provisions that we recommend in the event that the NIH does indeed, seek to execute an exclusive license for type 1 diabetes uses, for the patents noticed in 2018.

### **1. No discrimination against US residents in pricing**

We ask that the NIH include language in the proposed exclusive license to ensure that the prices in the U.S. for any drug, vaccine, medical device or other health technology using the inventions are not higher than the median price charged in the seven countries with the largest gross domestic product (GDP), that also have a per capita income of at least 50 percent of the United States, as measured by the World Bank Atlas Method.

We consider this a modest request to protect U.S. residents, who paid for the R&D that created the licensed inventions.

### **2. Reduce term of exclusivity when revenues are large**

In addition to an external reference pricing test, we propose that the exclusivity of the license in the U.S. should be reduced when the global cumulative sales from products or services using the inventions exceed certain benchmarks.

Given the modest cost of acquiring an NIH patented invention, the amount of money the developer needs in sales to justify additional investments in R&D is reduced, as compared to cases where a company develops or acquires the technology from non government sources.

This request is consistent with the statutory requirements of 35 USC § 209, which requires that “the proposed scope of exclusivity is not greater than reasonably necessary to provide the incentive for bringing the invention to practical application.”



One possible implementation of revenue benchmarks is as follows: exclusivity will be reduced by one year for every \$500 million in revenue equivalents, earned after the first \$1 billion, where revenue equivalent is defined as global cumulative sales plus market entry rewards as well as government grants or tax credits, for the product or products using the invention. However, the NIH could choose different benchmarks, so long as the limits on exclusivity address the requirements of 35 USC 209, that the incentive is “not greater than reasonably necessary.”

### **3. Developing countries**

We are concerned that several NIH-funded inventions are not accessible in developing countries, due to prices that are high and not affordable in markets where per capita incomes are significantly lower than the United States. For this reason, we ask the NIH to limit the exclusivity in the license to countries that have per capita incomes that are at least 30 percent of the United States.

We also ask the NIH to reach out to the Medicines Patent Pool (MPP), in order to enter into an agreement that gives the MPP an option to negotiate non-exclusive open licenses for the inventions in developing countries.

### **4. Transparency**

The licensee should be required to file an annual report to the NIH, available to the public, on the research and development (R&D) costs associated with the development of any product that uses the inventions, including reporting separately and individually the outlays on each clinical trial. We will note that this is not a request to see a company business plan or license application. We are asking that going forward the company be required to report on actual R&D outlays to develop the subject inventions. Reporting on actual R&D outlays is important for determining if the NIH is meeting the requirements of 35 USC § 209, that “the proposed scope of exclusivity is not greater than reasonably necessary to provide the incentive for bringing the invention to practical application.” Specifically, having data on actual R&D outlays on each clinical trial used to obtain FDA approval provides evidence that is highly relevant to estimating the risk adjusted costs of bringing NIH licensed inventions to market.

James Love  
james.love@keionline.org  
Director, Knowledge Ecology International (KEI)

Elizabeth Rowley  
elizabeth@t1international.com  
Director, T1International

KEI is an award-winning nonprofit organization that works extensively on issues pertaining to access to affordable medicines and related intellectual property concerns. KEI conducts research, writing, and advocacy in the public interest on behalf of patients, taxpayers, and consumers, including on the licensing of federally-funded and/or federally-owned medical technologies, and comments frequently on proposed exclusive licenses by the federal government including those by NIH.

T1International is a non-profit run by people with and impacted by type 1 diabetes for people with type 1 diabetes. T1International believe in a world where everyone with type 1 diabetes – no matter where they live – has everything they need to survive and achieve their dreams. T1International support local communities by giving them the tools they need to stand up for their rights so that access to insulin and type 1 diabetes supplies becomes a reality for all.

#### **Annex:**

#### **Patent documents listed in both the 2015 and 2018 Federal Register notices re: Inversago Pharma, Inc.**

<b>Application or patent code</b>	<b>HHS Reference number</b>	<b>Filing date</b>	<b>Patent office</b>
61/725,949	E-282-2012-0-US-01	11/13/2012	United States
PCT/US2013/069686	E-282-2012-0-PCT-02	11/13/2013	PCT
14/442,383 issued as 9,765,031	E-282-2012-0-US-03	11/13/2013	United States
2889697	E-282-2012-0-CA-04	04/27/2015	Canada
13802153.0	E-282-2012-0-EP-05	06/01/2015	EPO
3733/DELNP/2015	E-282-2012-0-IN-06	05/01/2015	India
2015-542015	E-282-2012-0-JP-07	05/11/2015	Japan
201380069389.9	E-282-2012-0-CN-08	07/03/2015	China
61/991,333	E-140-2014-0-US-01	05/09/2014	United States
62/171,179	E-282-2012-1-US-01	06/04/2015	United States
PCT/US2015/029946	E-140-2014-0-PCT-02	05/08/2015	PCT

---

**From:** Dodson, Sara (NIH/OD) [E] [/O=NIH/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=DODSONSE]  
**Sent:** 6/16/2017 8:20:35 PM  
**To:** Rohrbaugh, Mark (NIH/OD) [E] [/O=NIH/OU=NIH/EXCHANGE/cn=OD/cn=ROHRBAUM]  
**Subject:** RE: final memo to FC on royalties

Mark – the substantive edits are bolded & underlined below. Can you see the formatting?

**b5**

b5

**From:** Rohrbaugh, Mark (NIH/OD) [E]  
**Sent:** Friday, June 16, 2017 4:12 PM  
**To:** Dodson, Sara (NIH/OD) [E] <sara.dodson@nih.gov>  
**Subject:** Re: final memo to FC on royalties

Can you see his edits from the original?

Sent from my iPhone

On Jun 16, 2017, at 2:59 PM, Dodson, Sara (NIH/OD) [E] <sara.dodson@nih.gov> wrote:

Sure, Mark. I'm taking a look at this now.

**From:** Rohrbaugh, Mark (NIH/OD) [E]  
**Sent:** Friday, June 16, 2017 3:43 PM  
**To:** Dodson, Sara (NIH/OD) [E] <sara.dodson@nih.gov>  
**Cc:** Jorgenson, Lyric (NIH/OD) [E] <jorgensonla@od.nih.gov>  
**Subject:** Fwd: final memo to FC on royalties

Sorry to bug you late on Friday but could you try and edit this today based on my comments. I will send you Dale's edits separately

Sent from my iPhone

Begin forwarded message:

**From:** "Rohrbaugh, Mark (NIH/OD) [E]" <RohrBauM@OD.NIH.GOV>  
**Date:** June 16, 2017 at 2:28:40 PM CDT  
**To:** "Berkley, Dale (NIH/OD) [E]" <BerkleyD@OD.NIH.GOV>  
**Cc:** "Jorgenson, Lyric (NIH/OD) [E]" <jorgensonla@od.nih.gov>, "Hammersla, Ann (NIH/OD) [E]" <hammerslaa@mail.nih.gov>  
**Subject:** Re: final memo to FC on royalties

b5

The challenge to edit it is that I am traveling all day and can only work on my iPhone.

Sent from my iPhone

On Jun 16, 2017, at 3:09 PM, Berkley, Dale (NIH/OD) [E] <BerkleyD@OD.NIH.GOV> wrote:

REL0000024035



**b5**

Dale D. Berkley, Ph.D., J.D.  
Office of the General Counsel, PHD, NIH Branch  
Bldg. 31, Rm. 47  
Bethesda, MD 20892  
301 496 6043  
301-402-2528(Fax)

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**From:** Rohrbaugh, Mark (NIH/OD) [E]  
**Sent:** Friday, June 16, 2017 3:07 PM  
**To:** Berkley, Dale (NIH/OD) [E] <BerkleyD@OD.NIH.GOV>  
**Cc:** Jorgenson, Lyric (NIH/OD) [E] <jorgensonla@od.nih.gov>;  
Hammersla, Ann (NIH/OD) [E] <hammerslaa@mail.nih.gov>  
**Subject:** Re: final memo to FC on royalties

**b5**

Sent from my iPhone

On Jun 16, 2017, at 11:26 AM, Berkley, Dale (NIH/OD) [E]  
<BerkleyD@OD.NIH.GOV> wrote:

Attached are a few edits and comments for your consideration.

Best, Dale

Dale D. Berkley, Ph.D., J.D.  
Office of the General Counsel, PHD, NIH Branch  
Bldg. 31, Rm. 47  
Bethesda, MD 20892  
301 496 6043  
301 402 2528(Fax)

This message is intended for the exclusive use of the recipient(s) named above. It may contain information that is PROTECTED or PRIVILEGED, and it should not be disseminated, distributed, or copied to persons not authorized to receive such information.

**From:** Jorgenson, Lyric (NIH/OD) [E]  
**Sent:** Friday, June 16, 2017 10:07 AM

REL0000024035

**To:** Berkley, Dale (NIH/OD) [E]  
<[BerkleyD@OD.NIH.GOV](mailto:BerkleyD@OD.NIH.GOV)>  
**Cc:** Rohrbaugh, Mark (NIH/OD) [E]  
<[RohrBauM@OD.NIH.GOV](mailto:RohrBauM@OD.NIH.GOV)>  
**Subject:** FW: final memo to FC on royalties

Hi Dale,

Let me apologize in advance for being needy— I believe Mark Rohrbaugh sent you a copy of this draft response to KEI regarding CRISPR royalties. We are trying to get it under Francis' review today before Carrie leaves town. Did you have a chance to review?

Again – I apologize for the turn around.

Best,

Lyric

**From:** Rohrbaugh, Mark (NIH/OD) [E]  
**Sent:** Monday, May 15, 2017 3:19 PM  
**To:** Jorgenson, Lyric (NIH/OD) [E]  
<[jorgensonla@od.nih.gov](mailto:jorgensonla@od.nih.gov)>  
**Subject:** final memo to FC on royalties

Mark L. Rohrbaugh, Ph.D., J.D.  
Special Advisor for Technology Transfer  
Director, Division of Technology Transfer and  
Innovation Policy  
Office of Science Policy  
Office of the Director  
National Institutes of Health

<WF365656 KEI Response OEROSP (002)--  
OGCBerkleyComments.docx>

---

**From:** Hardesty, Rebecca (NIH/OD) [C] [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=21465CEB763947408D209AAABF10AF70-HARDESTYRS]  
**Sent:** 1/31/2018 11:46:00 AM  
**To:** Rohrbaugh, Mark (NIH/OD) [E] [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=591ab6b2424b4b8997082718cbb29fab-rohrbaum]; Jorgenson, Lyric (NIH/OD) [E] [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=3bbde7d361374981a4d336b6eeb17521-jorgensonla]  
**Subject:** Canceled: PMAP Review - Mark Rohrbaugh  
**Attachments:** Rohrbaugh 2017 Accomplishments.docx  
**Location:** 1/103  
  
**Start:** 2/5/2018 4:00:00 PM  
**End:** 2/5/2018 4:30:00 PM  
**Show Time As:** Free

**Required Attendees:** Rohrbaugh, Mark (NIH/OD) [E]; Jorgenson, Lyric (NIH/OD) [E]



Rohrbaugh 2017  
Accomplishment...

**b6**

**b6**

**b6**

---

**From:** Plude, Denise (NIH/OD) [E] [/O=NIH/OU=NIH/EXCHANGE/CN=RECIPIENTS/CN=PARKSDE]  
**Sent:** 5/3/2017 3:55:42 PM  
**To:** Rohrbaugh, Mark (NIH/OD) [E] [/O=NIH/OU=NIH/EXCHANGE/cn=OD/cn=ROHRBAUM]  
**CC:** Wertz, Jennifer (NIH/OD) [E] [/O=NIH/OU=NIH/EXCHANGE/cn=Recipients/cn=wertzj]  
**Subject:** WF 357204 - Necessary Action - due 5/4  
**Attachments:** DR01 Goldman Dir Sig 5.1.17.docx

**Work Folder Information**

**Work Folder:** WF 357204

**Process:** Necessary Action

**Program Analyst:** Hurllebaus, Lisa (NIH/OD) [E]

**Due Date:** May 04, 2017

**WF Subject:** OS assignment. KEI & UACT write about the prostate cancer drug, Xtandi (enzalutamide). Asks the Government to reconsider the decision not to use the 'march-in' rights, under the Bayh-Dole Act, for this excessively-priced drug. (AS-760889)

**IC:** od\_osp

**From:** Goldman, Andrew

**To:** Price, Tom; Mattis, Jim

**Remarks:** OER & OSP: OGC cleared the draft response with the following comments: "We have the following legal and non-legal comments."

b5

b5

b5

Please consider OGC's comments and rewrite, as appropriate, and submit in DDRMS. This is due by COB, tomorrow, Thursday, May 4, as we must meet OS's deadline of May 5 (and Drs. Tabak and Collins need to review). Thanks much, Lisa Hurllebaus



**b5**

---

**From:** Mowatt, Michael (NIH/NIAID) [E] [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=CB1EF7E2E54B4164AE34814574BDA638-MMOWATT]  
**Sent:** 11/17/2017 5:41:27 PM  
**To:** Rohrbaugh, Mark (NIH/OD) [E] [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=591ab6b2424b4b8997082718cbb29fab-rohrbaum]  
**Subject:** RE: NIAID responses to FRN comments

I'll set for 2:30.

Is (301) 435-4485 the best number?

---

**From:** Rohrbaugh, Mark (NIH/OD) [E]  
**Sent:** Friday, November 17, 2017 12:13 PM  
**To:** Mowatt, Michael (NIH/NIAID) [E] <mmowatt@niaid.nih.gov>  
**Subject:** RE: NIAID responses to FRN comments

Anytime after 1:30 works

**From:** Mowatt, Michael (NIH/NIAID) [E]  
**Sent:** Friday, November 17, 2017 12:09 PM  
**To:** Rohrbaugh, Mark (NIH/OD) [E] <rohrbaum@od.nih.gov>  
**Subject:** Fwd: NIAID responses to FRN comments

Close hold, please.

Can we discuss? 1230?

Begin forwarded message:

**From:** "Mowatt, Michael (NIH/NIAID) [E]" <mmowatt@niaid.nih.gov>  
**Date:** November 17, 2017 at 12:01:36 PM EST  
**To:** "Billet, Courtney (NIH/NIAID) [E]" <billetc@niaid.nih.gov>, "McGowan, John J. (NIH/NIAID) [E]" <jmcgowan@niaid.nih.gov>  
**Cc:** "Harper, Jill (NIH/NIAID) [E]" <jharper@niaid.nih.gov>, "Haskins, Melinda (NIH/NIAID) [E]" <haskinsm@mail.nih.gov>, "Stover, Kathy (NIH/NIAID) [E]" <kathy.stover@nih.gov>, "Frisbie, Suzanne (NIH/NIAID) [E]" <suzanne.frisbie@nih.gov>, "Mowatt, Michael (NIH/NIAID) [E]" <mmowatt@niaid.nih.gov>  
**Subject:** RE: NIAID responses to FRN comments

Thanks for your suggestions, Courtney.

**b5**

b5

b5

Please confirm that this is our plan.

**From:** Billet, Courtney (NIH/NIAID) [E]  
**Sent:** Friday, November 17, 2017 10:39 AM  
**To:** Mowatt, Michael (NIH/NIAID) [E] <[mmowatt@niaid.nih.gov](mailto:mmowatt@niaid.nih.gov)>; McGowan, John J. (NIH/NIAID) [E] <[jmcgowan@niaid.nih.gov](mailto:jmcgowan@niaid.nih.gov)>  
**Cc:** Harper, Jill (NIH/NIAID) [E] <[jharper@niaid.nih.gov](mailto:jharper@niaid.nih.gov)>; Haskins, Melinda (NIH/NIAID) [E] <[haskinsm@mail.nih.gov](mailto:haskinsm@mail.nih.gov)>; Stover, Kathy (NIH/NIAID) [E] <[kathy.stover@nih.gov](mailto:kathy.stover@nih.gov)>; Frisbie, Suzanne (NIH/NIAID) [E] <[suzanne.frisbie@nih.gov](mailto:suzanne.frisbie@nih.gov)>  
**Subject:** RE: NIAID responses to FRN comments

Hi Mike – I have two comments. One on content and the other on process.

b5

**From:** Mowatt, Michael (NIH/NIAID) [E]  
**Sent:** Friday, November 17, 2017 10:23 AM  
**To:** McGowan, John J. (NIH/NIAID) [E] <[jmcgowan@niaid.nih.gov](mailto:jmcgowan@niaid.nih.gov)>; Billet, Courtney (NIH/NIAID) [E] <[billetc@niaid.nih.gov](mailto:billetc@niaid.nih.gov)>  
**Cc:** Harper, Jill (NIH/NIAID) [E] <[jharper@niaid.nih.gov](mailto:jharper@niaid.nih.gov)>; Haskins, Melinda (NIH/NIAID) [E] <[haskinsm@mail.nih.gov](mailto:haskinsm@mail.nih.gov)>; Stover, Kathy (NIH/NIAID) [E] <[kathy.stover@nih.gov](mailto:kathy.stover@nih.gov)>; Frisbie, Suzanne (NIH/NIAID) [E] <[suzanne.frisbie@nih.gov](mailto:suzanne.frisbie@nih.gov)>; Mowatt, Michael (NIH/NIAID) [E] <[mmowatt@niaid.nih.gov](mailto:mmowatt@niaid.nih.gov)>  
**Subject:** NIAID responses to FRN comments

JJ and Courtney,

We have completed our diligence and plan to send the attached responses to ACOG and KEI/MSF. I'd like to get these out by Monday.

Please let me know if you have any questions, concerns, or suggestions for improving these documents.

Thanks,

Mike

**Michael R. Mowatt, Ph.D.**

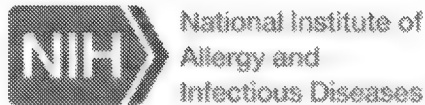
Director, Technology Transfer and Intellectual Property Office

**National Institute of Allergy and Infectious Diseases**

National Institutes of Health

U.S. Department of Health and Human Services

+1 301 496 2644



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---

**From:** Joe Allen [jallen@allen-assoc.com]  
**Sent:** 9/6/2017 9:20:15 PM  
**To:** Rohrbaugh, Mark (NIH/OD) [E] [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=591ab6b2424b4b8997082718cbb29fab-rohrbaum]; Robert Hardy [RHardy@COGR.edu]  
**Subject:** Interesting insight on march in rights and "reasonable terms"  
**Attachments:** Rabitschek and Latker on reasonable pricing and march in's.pdf

In getting ready for the meetings on the Hill tomorrow with BIO on the King/Sanders proposals, I was going through some materials including the attached law review article by Norm Latker and John Rabitschek. They make a really interesting point on p. 160.

They say that a "review of the statute makes it clear that the price charged by a licensee for a patented product has no direct relevance to march in rights." They look at the first march in provision on bringing the invention to practical application within a reasonable time-- which is where the fight on "reasonable pricing" occurs. Unlike the other march in triggers for availability to meet health/safety needs or to meet the requirements of Federal regulations, this provision on bringing the invention only applies to "the contractor or assignee" not the licensee as do the other two. That's accurate.

They then say because under most funding agreements, the contractor will be a university or nonprofit organization which is not engaged in product development, therefore:

"'Practical application.' as defined, requires the benefits of the invention be "available to the public on reasonable terms." With respect to a university patent owner, reasonableness would only apply to its licensing terms and to neither the price or availability of the licensed product. Further, in any license agreement, the price of the licensed product is left to the discretion of the licensee. Furthermore, if the license agreement were to specify a minimum sales price, this might constitute a violation of the antitrust laws. The typical license agreement includes a "due diligence" clause, so if the licensee is not adequately achieving commercialization, the university can terminate the license and seek other licensees."

What do you think of the argument that argument?

--

Joseph P. Allen  
President  
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(W) 740-484-1814  
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1-1-2005

# Reasonable Pricing - A New Twist for March-in Rights Under the Bayh-Dole Act

John H. Rabitschek

Norman J. Latker

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# REASONABLE PRICING – A NEW TWIST FOR MARCH-IN RIGHTS UNDER THE BAYH-DOLE ACT

**John H. Raubitschek†**

**Norman J. Latker††**

## INTRODUCTION

In 1980, the Bayh-Dole Act gave universities and small businesses the right to own their inventions created with federal funding.<sup>1</sup> Prior to this time, the existing statutes required certain agencies to own inventions arising from federally funded research.

The rationale of Bayh-Dole was simply this: if the law affords broad marketplace prerogatives to the developers of government funded inventions, the inventions are far more likely to be developed and so made available to the public. To achieve this goal, ownership is left with the innovators, rather than assigned to the government agency that financed the research. The innovators are then free to leverage their rights to their advantage, as intended by the patent system.

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† Patent Counsel, Department of Commerce, A.B. Princeton University, J.D. Georgetown Law Center. Member of the Bars of the District of Columbia and Virginia. The views expressed herein are those of the authors and not necessarily of the Department of Commerce or the U.S. Government.

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1. This is the popular name of the law, which takes its name from the principal sponsors in the Senate: Birch Bayh and Robert Dole. The actual name is the "University and Small Business Patent Procedures Act."



Although there was spirited opposition to Bayh-Dole when it was debated in Congress, a broad political consensus ultimately developed around the notion that market forces would do a better job of commercializing government-funded technology than federal agencies could.<sup>2</sup>

The Act has been enormously effective. As *The Economist: Science Technology Quarterly* concluded, the Act is “[p]ossibly the most inspired piece of legislation to be enacted in America over the past half-century.”<sup>3</sup> In operation, Bayh-Dole fostered a potent four-way partnership between researchers, their institutions, government, and industry. That partnership has created a powerful engine of practical innovation, producing many scientific advances that have extended human life, improved its quality and reduced suffering for millions of people.

Universities, in particular, have been very successful in commercializing their inventions. Bayh-Dole is generally credited for contributing to the dramatic increase over the last 25 years in the number of university inventions reported, patents granted, royalty-bearing licenses negotiated, collaborative research agreements signed, and start-up companies founded. As noted by *The Economist*, since 1980, American universities have witnessed a ten-fold increase in their patents and created more than 2,200 companies to exploit their technology, which in turn has produced 260,000 new jobs; they now contribute \$40 billion annually to the American economy.<sup>4</sup>

Notwithstanding its unquestioned success, the Act has recently been criticized on the basis that the public should not be charged, or should be charged less, for goods based on inventions for which, the opponents maintain, the taxpayers have already paid.<sup>5</sup> There have

2. S. 414, 96th Cong. (1980). The bill passed the Senate 91-4 and resulted in the Bayh-Dole Act, Pub. L. 96-517, 96th Cong., 94 Stat. 3015 (1980). The agencies’ passive licensing approach resulted in less than 4% of government patents being licensed as of 1975 in comparison with 33% of university owned licensed patents. S. Rep. No. 96-480, at 2 (1979).

3. The Economist Newspaper and the Economist Group, *Innovation’s Golden Goose*, THE ECONOMIST: SCIENCE TECHNOLOGY QUARTERLY (2002), available at [http://www.economist.com/science/tq/displayStory.cfm?story\\_id=1476653](http://www.economist.com/science/tq/displayStory.cfm?story_id=1476653) (last visited May 31, 2005).

4. *Id.*

5. This criticism is remarkably similar to the views of some opponents of Bayh-Dole. See *The University and Small Business Patent Procedures Act: Hearing on S. 414 before the Senate Comm. on the Judiciary*, 96th Cong. 157 (1979) (statement of Admiral Hyman Rickover) [hereinafter *University Hearing*]. Admiral Rickover states “[i]n my opinion, Government contractors—including small businesses and universities—should not be given title to inventions developed at Government expense . . . . These inventions are paid for by the public and therefore should be available for any citizen to use or not as he sees fit.”

been an increasing number of articles expressing this view and further suggesting that Bayh-Dole was not intended to give innovators an unfettered right to set market prices for their inventions, which has contributed to the rising cost of health care, especially for patented drugs.

One such article by Peter S. Arno and Michael H. Davis asserts that “march-in rights” were clearly intended to combat the price of drugs invented by universities with federal funds and identified to be excessive.<sup>6</sup> It is the purpose of this article to analyze this assertion and its consequences.

## I. HISTORY OF MARCH-IN RIGHTS

### A. 1947 Attorney General Report

March-in rights have a long history and were discussed in the 1947 Attorney General’s Report and Recommendations to the President.<sup>7</sup> They were included in the proposed government patent policy which was being developed to accompany the expansion of government research and development programs after World War II,

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6. Peter Arno & Michael Davis, *Why Don't We Enforce Existing Drug Price Controls? The Unrecognized and Unenforced Reasonable Pricing Requirements Imposed upon Patents Deriving in Whole or in Part from Federally Funded Research*, 75 TULANE L. REV. 631 (2001). Arno and Davis presented similar arguments in an op-ed article. See Peter Arno & Michael Davis, *Paying Twice For the Same Drugs*, WASHINGTON POST, March 27, 2002, at A21. This was rebutted by Birch Bayh and Robert Dole in another op-ed article. See Birch Bayh and Robert Dole, *Our Law Helps Patients Get New Drugs Sooner*, WASHINGTON POST, April 11, 2002, at A28, stating:

Bayh-Dole did not intend that government set prices on resulting products. The law makes no reference to a reasonable price that should be dictated by the government . . . . The [Arno and Davis] article also mischaracterizes the rights retained by the government under Bayh-Dole. The ability of the government to revoke a license granted under the act is not contingent on the pricing of the resulting product or tied to the profitability of a company that has commercialized a product that results in part from government-funded research. The law instructs the government to revoke such licenses only when the private industry collaborator has not successfully commercialized the invention as a product.

7. U.S. Dept of Justice, INVESTIGATION OF GOVERNMENT PATENT PRACTICES AND POLICIES, REPORT AND RECOMMENDATIONS OF THE ATTORNEY GENERAL TO THE PRESIDENT (1947) [hereinafter RECOMMENDATIONS]. There are three volumes. The report was initiated by a letter dated February 5, 1943 from President Franklin Delano Roosevelt to Attorney General Francis Biddle. President Roosevelt felt there was a need for a uniform Government policy on the ownership of inventions made by Government employees and contractors.

as recommended by the presidential science adviser, Vannevar Bush.<sup>8</sup> The Attorney General's Report recommended that, generally, the Government should own inventions made by contractors, but that in special circumstances the contractor may be permitted to own its inventions, provided that "[t]he contractor (or his assignee) shall be required to offer nonexclusive licenses at a reasonable royalty to all applicants" if the contractor or assignee does not place the invention in adequate commercial use within a designated period.<sup>9</sup>

### *B. 1963 and 1971 Presidential Memoranda and Statements*

Thereafter, similar provisions attached to contractor ownership of inventions were described in the Presidential Memoranda and Statements of Government Patent Policy by Presidents Kennedy (1963) and Nixon (1971).<sup>10</sup> These were implemented in the Federal Procurement Regulations and various agency procurement regulations.<sup>11</sup>

#### **1. The Kennedy Memorandum**

According to section 1(f) of the Kennedy Memorandum, the government shall have the right to require the granting of a nonexclusive royalty-free license to an applicant if the contractor or grantee who has been permitted to own the invention, or its licensee or assignee, (1) has not taken effective steps within three years after the patent issues to bring the invention to the point of practical

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8. Vannevar Bush, *Science: The Endless Frontier*, (U.S. GOV'T PRINTING OFFICE July 1945), available at <http://www.nsf.gov/od/lpa/nsf50/vbush1945.htm> (last visited May 31, 2005) (report to the President on a program for postwar scientific research).

9. RECOMMENDATIONS, *supra* note 7, vol. 1 at 76, 110.

10. Memorandum of October 10, 1963, 28 Fed. Reg. 10,943 (Oct. 12, 1963) [hereinafter Kennedy Memorandum]. Memorandum of August 23, 1971, 36 Fed. Reg. 16,887 (Aug. 26, 1971) [hereinafter Nixon Memorandum].

11. Federal Procurement Regulations § 1-9.107-3(b), 38 Fed. Reg. 23,782 (Sept. 4, 1973), revised by 40 Fed. Reg. 19,814 (May 7, 1975) (codified at 37 C.F.R. § 401.14 and 48 C.F.R. § 52.227-11). Compare with Federal Nonnuclear Act of 1974 § 9(h), 42 U.S.C. § 5908(h)(6) (2000) (repealed). A march-in like provision which allowed the head of the agency to terminate a waiver of title or grant of an exclusive license if the recipient has not taken effective steps necessary to accomplish substantial utilization of the invention. Section 9 was later repealed by Bayh-Dole.) See discussion of march-in by the Department of Energy in GAO letter to Senator Bayh dated July 17, 1979, reprinted in *University Hearing*, *supra* note 5, at 52-56. According to DOE as reported by GAO, march-in was intended to address contractor windfall profits but has not been utilized although available for more than 10 years because the problems are illusory and not actual. *Id.* at 56.

application,<sup>12</sup> or (2) has made the invention available for licensing royalty free or on terms that are reasonable in the circumstances, or (3) can show why it should be able to retain ownership for a further period of time.<sup>13</sup> As in the Attorney General's Report, the fourth paragraph of the Kennedy Memorandum made clear that the reason for march-in rights was to "guard against failure to practice the invention."<sup>14</sup>

## 2. The Nixon Memorandum

The march-in rights in section 1(f) of the Nixon Memorandum are essentially the same as those in the Kennedy Memorandum,<sup>15</sup> except that the requirement was expanded to assignees and licensees and the Government could also require the granting of an exclusive license to a responsible applicant on terms that were reasonable under the circumstances if the invention was not being developed.

Arno and Davis note that both Presidential Memoranda require that licensing of inventions be on "reasonable terms." There is no requirement in the Memoranda that the price of a patented invention be on "reasonable terms."

## C. Institutional Patent Agreements

Institutional Patent Agreements (IPAs) were first used by the National Institutes of Health (NIH) beginning in 1968 and later by the National Science Foundation (NSF) in 1973 to govern the management of inventions made with NIH/NSF support by universities with an approved patent policy. Since many of the provisions in the Bayh-Dole Act come from IPAs,<sup>16</sup> Bayh-Dole can

12. "To the point of practical application" is defined as: "to manufacture in the case of a composition or product, to practice in the case of a process, or to operate in the case of a machine and under such conditions as to establish that the invention is being worked and that its benefits are reasonably accessible to the public." Kennedy Memorandum, *supra* note 10, § 3(g).

13. *Id.* § 1(f). Refers to principal or exclusive rights rather than ownership because of the required irrevocable paid-up license for government purposes throughout the world.

14. *Id.*

15. Nixon Memorandum, *supra* note 10, § 1(f). The definition of "to the point of practical application" was unchanged. *Id.* § 4(g).

16. There are a number of common elements: (1) restriction against assignment of inventions except to a patent management organization, (2) limitation on the term of an exclusive license, which was removed when Bayh-Dole was amended in 1984, (3) requirement that royalty income must be shared with inventors and the remainder used for education and research purposes, (4) requirement that any patent application contain a reference to the federal support which resulted in the invention and (5) requirement of granting a paid-up license to the Government.



be considered a codification of the IPA. Under both the NIH and NSF IPAs, as in Bayh-Dole, the university had a contractual right to elect ownership to any invention, thereby eliminating the arduous task of justifying ownership after identification of an invention. Each IPA contained all the conditions required by the Presidential Memoranda, including march-in rights and the requirement to license on "reasonable terms."

After receiving comments from many agencies and universities, a model IPA containing these conditions was later developed for government-wide use by the University Patent Policy Ad Hoc Subcommittee of the Committee on Government Patent Policy of the Federal Council of Science and Technology.<sup>17</sup> Implementation of the model IPA was postponed for 120 days at the request of Senator Gaylord Nelson on March 17, 1978, who held hearings,<sup>18</sup> and sought to receive recommendations by July 18, 1978.<sup>19</sup>

#### *D. Use of March-In Prior to 1980*

Before Bayh-Dole, there was little activity regarding march-in rights.<sup>20</sup> At most, the focus was on whether a particular invention funded by the Government was being used. The absence of march-in rights was discussed during the Nelson hearings. In particular, Donald R. Dunner, the first Vice President of the American Patent Law Association, indicated that:

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17. The Subcommittee was chaired by Norman Latker and included John Raubitschek, then patent counsel for NSF, as a member.

18. *Government Patent Policies: Institutional Patent Agreements: Hearings before the Subcommittee on Monopoly and Anticompetitive Activities of the Senate Select Committee on Small Business*, 95th Cong. 4 (1978), [hereinafter *IPA Hearings*].

19. *Id.* at 1004.

20. See *Patent Policy: Hearings on S. 1215 Before the Senate Subcomm. on Science, Technology and Space of the Comm. on Commerce, Science, and Transportation*, 96th Cong. 366 (1979), where Dale Church of the Department of Defense responded to Senator Stevenson's question, "Has the Department exercised march-in rights?", by remarking, "Only once can I recall there was a case where we exercised march-in rights. It was a case involving two patents held by MIT. There was a complainant who felt as though the patents were not being utilized. As to one of the patents, it was found that MIT was using it and was allowed to retain exclusive title. In the case of the other, we found that MIT was not effectively using it, and they did provide for the complainant to use the patent." See also Lynn J. Alstadt, *The 1980 Patent Rights Statute: A Key to Alternate Energy Sources*, 43 U. PITT. L. REV. 73, 95 n.121 (1981), which discusses march-in activity at NIH, NSF and the Air Force, and Diane M. Sidebottom, *Intellectual Property in Federal Government Contracts: The Past, The Present and One Possible Future*, 33 PUB. CONT. L.J. 63, 95 n.245 (2003), which refers to two march-ins by the predecessor to the Department of Energy in 1974. But these may have related to "waived" inventions. See Federal Nonnuclear Act, *supra* note 11.

Much has been said about march-in rights . . . . The point has been raised that march-in rights have been available for 10 years, and they have never been used; ergo, they are a failure. We submit that is not the case. There is no evidence to indicate that march-in rights should have been used in a specific situation and were not used. In fact, we submit the high probability is quite the contrary. Where an invention is significant, we submit that the marketplace will take care of the situation. Competitors who want to use a given piece of technology follow a standard routine procedure. They first determine whether there is any patent cover on the development, and then they evaluate the patent cover. If they feel they want to get into the field, they will try to get a license. If they cannot get a license in a Government-owned situation, they will go to the Government agency involved, and they will say, 'I cannot get a license.' They will point to the conditions which the IPA specify as to when march-in rights should be applied; they will provide the information necessary for that evaluation to be made, and we submit in any given situation where march-in should be applied, they will be applied.<sup>21</sup>

## II. MARCH-IN RIGHTS UNDER BAYH-DOLE

Under Bayh-Dole, the Government's march-in rights are described in 35 U.S.C. § 203. The funding agency may take action if the contractor, grantee, or assignee has not taken, or is not expected to take within a reasonable time, effective steps to achieve practical application in a field of use.<sup>22</sup> This was clearly intended to follow the precedent established in both Presidential Memoranda and the IPAs. "Practical application" is defined in 35 U.S.C. § 201(f) to mean:

[T]o manufacture in the case of a composition or product, to practice in the case of a process or method, or to operate in the case of a machine or system; and, in each case, under such conditions as to establish that the invention is being utilized and

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21. *IPA Hearings*, *supra* note 18, at 577.

22. *See generally* 35 U.S.C. § 203 (2000). It is interesting that § 203 does not mention "licensee" in addition to contractor, grantee, or assignee, as did the Nixon Memorandum and so does not directly consider the commercialization activities of the contractor's licensee. There are three other bases for exercising march-in rights. *Id.* § 203(1)(a). Two relate to health, safety or public use and so are similar to the Nixon Memorandum except that they come into play only if the contractor, grantee, assignee or licensee cannot reasonably alleviate or satisfy such needs. The third basis relates to a breach of the "domestic manufacturing" requirement in 35 U.S.C. § 204 (2000). *See* 35 U.S.C. § 203(1)(b)-(d) (2000).

that its benefits are to the extent permitted by law or Government regulations available to the public on reasonable terms.<sup>23</sup>

Section 203 not only authorizes the funding agency to require the contractor or grantee, or its assignee or exclusive licensee, to grant a license to a responsible applicant but itself can grant a license if the ordered party refuses to do so.<sup>24</sup>

According to the legislative history of Bayh-Dole:

The Government may "march-in" if reasonable efforts are not being made to achieve practical application, for alleviation of health and safety needs, and in situations when use of the invention is required by Federal regulations. "March-in" is intended as a remedy to be invoked by the Government and a private cause of action is not created in competitors or other outside parties, although it is expected that in most cases complaints from third parties will be the basis for the initiation of agency action.<sup>25</sup>

Any decision to exercise march-in is subject to appeal to the Court of Federal Claims within 60 days. The agency's decision is held in abeyance until all appeals are exhausted. A decision not to exercise rights is not reviewable.<sup>26</sup>

The Bayh-Dole regulation in 37 C.F.R § 401.6 sets forth a detailed multi-step process, although the agency can terminate the proceedings at any time.<sup>27</sup> The regulation allows an agency to initiate a march-in proceeding "[w]henever [it] receives information that it believes might warrant the exercise of march-in rights."<sup>28</sup> Since the regulation provides no criteria for the initiation of a proceeding, an agency appears to have unlimited discretion on whether or not to

23. This definition differs from those of the Kennedy and Nixon Memoranda, which say merely "that its benefits are reasonably accessible to the public."

24. Licensing by the Government would be unusual since it is not the patent owner. If there were royalties, it is assumed that they would belong to the patentee or exclusive licensee.

25. S. REP. NO. 96-480, *supra* note 2 at 33-34.

26. *Id.* at 34.

27. 37 C.F.R § 401.6(h) (2004). Thus, one author has concluded that the procedures have a built-in asymmetry which discourages march-in. See Avital Bar-Shalom and Robert Cook-Deegan, *Patents and Innovation in Cancer Therapeutics: Lessons from CellPro*, 80 THE MILBANK QUARTERLY 637, 667 (2002):

The procedures stipulated in Bayh-Dole also have a built-in asymmetry that discourages march-ins. If an agency decides not to march in, the case is over. If it does decide to march in, the party whose patent is subject to compulsory licensing can contest the decision, which compels the agency to defend its action against a party with a strong financial stake.

28. 37 CFR § 401.6(b) (2004).



initiate one.<sup>29</sup> However, before initiating a proceeding, the agency is required to notify the contractor and request its comments.<sup>30</sup>

Since 1980, the government has not exercised march-in rights.<sup>31</sup> This might be an indication that march-in is simply ineffective.<sup>32</sup> The ineffectiveness is demonstrated by the discovery made by the Government Accountability Office (GAO), which pointed out that agencies do not seek commercialization reports from contractors and so do not know if inventions are being commercialized.<sup>33</sup> Nevertheless, there have been three petitions to the Department of Health and Human Services (HHS) in recent years.

On March 3, 1997, CellPro, Inc. asked HHS to march-in against Johns Hopkins University. The matter involved Johns Hopkins' exclusive licensee Baxter Healthcare Corporation on four patents covering an antibody useful for the treatment of cancer (U.S. Patent Nos. 4,965,204, 4,714,680, 5,035,994 and 5,130,144).<sup>34</sup> The petition was referred to NIH, which funded the research resulting in the inventions. Dr. Harold Varmus, the Director of NIH, concluded that march-in proceedings were not warranted in a decision dated August 1, 1997.<sup>35</sup> Dr. Varmus argued that march-in proceedings were not necessary because Baxter Healthcare Corporation, an exclusive

29. Failure to enforce a statute is presumptively discretionary and therefore unreviewable under the Administrative Procedure Act. *Heckler v. Chaney*, 470 U.S. 821, 837-38 (1985). However, Arno & Davis, *supra* note 6, 689-90 n.366, suggested that an argument could be made that the detailed requirements in 35 U.S.C. § 202 (2000) amount to the kind of guidelines that would render the agencies' actions reviewable.

30. 37 CFR § 401.6(b) (2004).

31. Several authors have suggested that the Government will never exercise these rights. See generally Bar-Shalom and Cook-Deegan, *supra* note 27 and Kevin W. McCabe, *Implications of the CellPro Determination on Inventions Made with Federal Assistance: Will the Government Ever Exercise Its March-in Rights?*, 27 PUB. CONTR. L.J. 645 (1998). See also *University Hearing*, *supra* note 5, at 160 (Admiral Rickover, no supporter of the Bayh-Dole Act, considered that march-in as a safeguard was "largely cosmetic" because in the rare case of an agency exercising march-in, it would take years of litigation).

32. To the contrary, Mr. Dunner has suggested that the lack of any march-in by an agency does not mean it is a failure because there is no evidence of when it should have been used and that the marketplace would take care of the need for march-in with significant inventions. See *University Hearing*, *supra* note 5, at 577.

33. TECHNOLOGY TRANSFER: REPORTING REQUIREMENTS FOR FEDERALLY SPONSORED INVENTIONS NEED REVISION, GAO/RCED-99-242, at 15-16 (U.S. General Accounting Office 1999).

34. Peter Mikhail, *Hopkins v. CellPro: An Illustration That Patenting and Exclusive Licensing of Fundamental Science Is Not Always in the Public Interest*, 13 HARVARD J.L. TECH. 375, 385 (2000).

35. Determination In the Case of Petition of CellPro, Inc. (National Institutes of Health August 1, 1997), available at <http://www.nih.gov/news/pr/aug97/nihb-01.htm> (last visited September 1, 2005) [hereinafter Cellpro Determination].

licensee, had taken steps to make its product available to the public on reasonable terms by obtaining European approval and filing for FDA approval. Dr. Varmus also noted that it would be inappropriate for NIH "to procure for CellPro more favorable commercial terms that it can otherwise obtain from the Court or from the patent owners."<sup>36</sup> This matter was complicated by the pending patent infringement suit by Johns Hopkins University against CellPro filed in 1994, and included appeals to the Federal Circuit, which ultimately sustained the validity and infringement of the Hopkins patents.<sup>37</sup>

On January 29, 2004, James Love and Sean Flynn filed two march-in petitions to HHS on behalf of Essential Inventions, Inc., relying on the Arno-Davis "reasonable pricing" theory.<sup>38</sup> Both petitions were referred to NIH, which had funded the research resulting in the two patented inventions.

One petition related to ritonavir, a drug for the treatment of AIDS sold under the trade name of Norvir® and invented by Abbott Laboratories under a \$3.5 million grant from the National Institute for Allergy and Infectious Diseases (NIAID) (U.S. Patent No. 6,232,333).<sup>39</sup> There were other Abbott patents (U.S. Patent Nos. 5,541,206, 5,635,523, 5,648,497, 5,674,882, 5,846,987 and 5,886,036) relating to specific formulations or delivery techniques for Norvir®, which may not have been invented under the NIAID grant.

The petition appears to have been a reaction to Abbott's increasing the U.S. retail price of Norvir® by 400% in December 2003, when it shifted from being a primary treatment agent to one used in small doses to boost the effects of other anti-AIDS medicines. Norvir® has been a very successful drug, with total sales of more than

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36. *Id.* For a description and analysis of the Cellpro case by two NIH attorneys, see Barbara M. McGarey & Annette C. Levey, *Patents, Products, and Public Health: An Analysis of the CellPro March-In Petition*, 14 BERKELEY TECH. L.J. 1095 (1999). See also Tamsen Valoir, *Government Funded Inventions: The Bayh-Dole Act and the Hopkins v. Cellpro March-In Rights Controversy*, 8 TEX. INTELL. PROP. L.J. 211, 219-33 (2000). There has been some criticism of the CellPro decision. See Bar-Shalom & Cook-Deegan, *supra* note 27; McCabe, *supra* note 31; and Mikhail, *supra* note 34.

37. *Johns Hopkins Univ. v. CellPro, Inc.*, 152 F.3d 1342 (Fed. Cir. 1998).

38. See *Essential Inventions, Inc. Legal Documents*, available at <http://www.essentialinventions.org/legal> (last visited September 1, 2005). Both petitions requested that HHS issue non-exclusive licenses on the same non-discriminatory terms but suggested that each patent owner receive a 5% royalty from the generic drug companies.

\$1 billion since it was introduced, although sales fell to \$100 million in 2003 from a high of \$250 million in 1998.<sup>40</sup>

A public meeting was held at NIH on May 25, 2004, to discuss the petition on the patents on Norvir® owned by Abbott Laboratories. Norman Latker, James Love, and former Senator Birch Bayh, one of the principal co-sponsors of Bayh-Dole, as well as a number of other people from universities and the private sector, spoke on the issue.<sup>41</sup>

In a decision dated July 29, 2004 and released on August 4, 2004, Dr. Elias Zerhouni, the Director of NIH, determined that NIH did "not have information that leads it to believe that the exercise of march-in rights might be warranted."<sup>42</sup> NIH found that the record established that Abbott had met the standard for achieving practical application by its manufacture, practice and operation of Norvir®, by the drug's availability and use by patients with HIV/AIDS since 1996, along with Abbott's active marketing. With respect to drug pricing, NIH felt "that the extraordinary remedy of march-in is not an appropriate means of controlling prices . . . [which should be] left for Congress to address legislatively." Further, any anti-competitive behavior by Abbott should be addressed by the FTC. Essential Inventions responded on August 4, 2004 disagreeing with NIH's decision: "The plain language of the Bayh-Dole Act says that government-funded inventions should be made 'available to public on reasonable terms.'"<sup>43</sup>

The other petition related to latanoprost, a drug for the treatment of ocular hypertension and glaucoma sold under the trade name of Xalatan®, invented by Columbia University under a grant from the National Eye Institute, and exclusively licensed to Pharmacia Corporation, now owned by Pfizer (U.S. Patent No. 4,599,353).<sup>44</sup>

40. Associated Press, *U.S. Won't Override AIDS Drug Patents*, U.S.A. TODAY, Aug. 4, 2004, available at [http://www.usatoday.com/news/washington/2004-08-04-aids-drug\\_x.htm](http://www.usatoday.com/news/washington/2004-08-04-aids-drug_x.htm) (last visited September 1, 2005).

41. Press Release, Essential Inventions, Inc., Essential Inventions Responds to NIH Refusal to Authorize Generic Versions of Overpriced AIDS Drug (Aug. 4, 2004), available at <http://www.essentialinventions.org/drug/nih05252004> (last visited September 4, 2005) [hereinafter Press Release].

42. In the Case of NORVIR® Manufactured by Abbott Laboratories, Inc. (National Institutes of Health July 29, 2004) (determination) available at <http://OTT.od.nih.gov/Reports/March-In-Norvir.pdf> (last visited September 1, 2005).

43. Essential Inventions Responds to NIH Refusal to Authorize Generic Versions of Overpriced AIDS Drug (August 4, 2004), available at <http://www.essentialinventions.org/drug/august42004pressrelease.htm> (last visited September 4, 2005).

44. It is of interest that Arno & Davis, *supra* note 6 at 689, mentioned this drug as one where there should have been price controls. An extensive history of this drug is provided by

Pfizer owns at least three other U.S. patents (5,296,504, 5,422,368 and 6,429,226) relating to Xalatan®, none of which were made with federal funds and so are not subject to march-in. According to the petition, Pfizer sells Xalatan® in the United States for two to five times the price charged in Canada and Europe. The drug is said to cost as much as \$65 for a four to six week supply, although the cost of the active ingredient is less than 1% of the sales price. By 2000, the sales of Xalatan® were over \$500 million a year. The petition considered this unreasonable in view of over \$4 million of taxpayer support for the research at Columbia University.

In a decision by Dr. Zerhouni dated September 17, 2004, the National Institutes of Health “determined that it will not initiate a march-in proceeding as it does not believe such a proceeding is warranted based on the available information and the statutory and regulatory framework.”<sup>45</sup> The basis for the decision was that the record “demonstrates that Pfizer has met the standard for achieving practical application of the applicable patents by its manufacture, practice, and operation of latanoprost and the drug’s availability and use by the public.” With respect to the lower prices being charged in Canada and Europe, NIH “believes that the extraordinary remedy of march-in is not an appropriate means for controlling prices.” Rather, NIH felt that the lower foreign prices should be “appropriately left for Congress to address legislatively.”

#### A. “Reasonable Terms” Relate to Licensing

A review of the statute makes it clear that the price charged by a licensee for a patented product has no direct relevance to march-in rights. As set forth in 35 U.S.C. § 203(1)(a), the agency may initiate a proceeding if it determines that the contractor or assignee has not taken, or is not expected to take within a reasonable time, effective steps to achieve practical application of an invention made under the contract.<sup>46</sup> In most funding agreements, the contractor will be a

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Jeff Gerth & Sheryl Gay Stolberg, *Drug Makers Reap Profits on Tax-Backed Research*, N.Y. TIMES, April 23, 2000, at A1. According to this article, when the patent application was filed in 1982, no drug company in the United States was interested in a license because of its unusual approach to treating glaucoma.

45. In the Case of Xalatan® Manufactured by Pfizer, Inc. (National Institutes of Health Sept. 17, 2004) (determination), available at <http://OTT.od.nih.gov/Reports/March-in-xalatan.pdf> (last visited August 31, 2005).

46. Under 35 U.S.C. § 202(c)(7) (2000), a university is not permitted to assign its invention without the approval of the agency except to a patent management organization.



university or nonprofit organization. Under the law, the university need only take "effective steps," not achieve practical application.<sup>47</sup>

If a university is not directly engaged in the development of its invention, an agency should inquire as to what steps the university is planning on taking to commercialize the invention in a reasonable time. Since this involves future action and an undefined time period, it is not clear how an agency would evaluate this.<sup>48</sup> On the other hand, if the university has licensed a company to make, use and sell the invention, it may be considered as having taken effective steps even if no sales of the invention have yet to occur, assuming that the licensee is making some efforts to commercialize the invention.<sup>49</sup>

"Practical application," as defined, requires the benefits of the invention be "available to the public on reasonable terms." With respect to a university patent owner, reasonableness would apply only to its licensing terms and to neither the price nor the availability of the licensed product.<sup>50</sup> Further, in any license agreement, the price of the licensed product is left to the discretion of the licensee.<sup>51</sup> Furthermore, if the license agreement were to specify a minimum sales price, this might constitute a violation of the antitrust laws. The typical license agreement includes a "due diligence" clause, so if the licensee is not adequately achieving commercialization, the university can terminate the license and seek other licensees.

With Norvir®, Abbott Laboratories, not a university, was the contractor and so was directly responsible for commercialization of that invention. Since there was no license, there was no issue of "reasonable terms," and the dramatic price increase in Norvir® and the substantial funding of the research by NIH were not relevant.<sup>52</sup>

47. 35 U.S.C. § 203(1)(a) (2000).

48. Under both the Presidential Memoranda, the time period was three years from the issue date of the patent. See Kennedy Memorandum, *supra* note 10, and Nixon Memorandum, *supra* note 10. A mere statement that a patent is available for licensing may not be sufficient.

49. See CellPro Determination, *supra* note 35.

50. But in the CellPro march-in case, NIH interestingly concluded that practical application had been achieved because the licensee was manufacturing, practicing and operating the licensed product. See McGarey & Levey, *supra* note 36, at 1101. Of course, in view of the substantial sales of Xalatan®, the benefits of this invention would have been reasonably available to the public under this approach.

51. The model IPA contained a requirement that royalties "be limited to what is reasonable under the circumstances or within the industry involved." Thus, the focus of reasonable terms was on the licensing by the universities and not the price of the licensed product.

52. Essential Inventions, Inc. filed a complaint with the Federal Trade Commission on January 29, 2004, alleging that the 400% increase in price for Norvir® on December 2003 constituted anti-competitive pricing practices and thus violated antitrust laws. Letter from

Rather, since Norvir® is available to the public from Abbott, either directly or through other companies that can purchase it from Abbott, there was no basis to conduct a march-in rights proceeding under 35 U.S.C. § 203(1)(a).<sup>53</sup> By manufacturing and selling Norvir®, Abbott has taken effective steps to achieve practical application. According to the petition, the sales of Norvir® through 2001 have totaled more than \$1 billion and may reach \$2 billion over the next ten years.

*B. There is No Reasonable Pricing Requirement*

Arno and Davis maintain that “[t]he requirement for ‘practical application’ seems clear to authorize the federal government to review the prices of drugs developed with public funding under Bayh-Dole terms and to mandate march-in when prices exceed a reasonable level.”<sup>54</sup> Arno and Davis further suggest that under Bayh-Dole, the contractor may have the burden of showing that it charged a reasonable price.<sup>55</sup> This could be made part of its business development or marketing plan.<sup>56</sup>

As we have mentioned previously, there is very little legislative history on march-in rights and nothing relating to when they are to be used. Similarly, Arno and Davis acknowledge that there is no clear legislative history on the meaning of the phrase “available to the public on reasonable terms,”<sup>57</sup> yet they conclude that “[t]here was

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Essential Inventions, Inc., to Susan Creighton, Director, Bureau of Competition – Federal Trade Commission (January 29, 2004), *available at*

<http://www.essentialinventions.org/legal/norvir/ftcletter.pdf> (last visited September 1, 2005). On May 19, 2004, Senators Charles Schumer, John McCain and Fritz Hollins asked the FTC to initiate an investigation into Abbott’s sudden price increase for Norvir®. See Letter from Schumer et al., United States Senate, to Timothy J. Muris, Chairman, Federal Trade Commission (May 19, 2004), *available at*

<http://www.essentialinventions.org/legal/norvir/schmccoll2FTC051904.pdf> (last visited September 1, 2005). The FTC later advised Abbott that it had no plans to investigate this complaint. See Associated Press, *supra* note 40. Dr. Jeffrey Leiden, president of Abbot, commented at the NIH public meeting on May 25, 2004, that the NIH funding of the invention was around \$3.5 million. Jeffrey M. Leiden, *Abbot Laboratories Comments at NIH Public Meeting Regarding Norvir® and Bayh-Dole March-in Provisions* (May 25, 2004), *available at* <http://www.essentialinventions/drug/nih05252004/leiden.pdf> (last visited June 29, 2005).

53. But see 35 U.S.C. § 203(1)(b) (2000), the march-in for health and safety needs.

54. Arno & Davis, *supra* note 6, at 651.

55. *Id.* at 653.

56. There is no requirement in Bayh-Dole for contractors to have such a plan although there is one for Federal laboratories in 35 U.S.C. § 209 (2000). In 2000, Congressman Sanders offered an amendment to HHS appropriations bill H.R. 4577 that would apply the licensing requirements for Federal laboratories to universities. See discussion of Sanders’ amendment in Arno & Davis, *supra* note 6, at 635 n.12, 666-67 n.227. The amendment was not adopted.

57. Arno & Davis, *supra* note 6, at 649.

never any doubt that this meant the control of profits, prices, and competitive conditions.”<sup>58</sup>

Support for this surprising conclusion that “reasonable terms” means “reasonable prices” is found in unrelated testimony during the Bayh-Dole hearings, and in other Government patent policy bills that did not become law, as well as in a number of non-patent regulatory cases.<sup>59</sup> Even if “reasonable terms” is interpreted to include price that does not necessarily mean that patented drugs funded by the Government must be sold at reasonable prices.

If Congress meant to add a reasonable pricing requirement, it would have explicitly set one forth in the law, or at least described it in the accompanying reports.<sup>60</sup> That a new policy could arise out of silence would truly be remarkable. There was no discussion of the shift from the “practical application” language in the Presidential Memoranda and benefits being reasonably available to the public, to benefits being available on reasonable terms under 35 U.S.C. § 203.

On the other hand, there was much debate during the Bayh-Dole hearings on whether there should be a recoupment provision to address any windfall profits that a university may make out of research funded by the Government.<sup>61</sup> There was a recoupment provision in S. 414 as passed by the Senate but it did not become law.<sup>62</sup> Further, the pre-1984 limitation on the length of an exclusive license term in Bayh-Dole meant that other companies would have access to the patented technology after five years from the first commercial sale or eight years from the date of license.<sup>63</sup>

After convincing themselves that they have made their case, Arno and Davis criticize Bayh-Dole and the Department of Commerce’s implementing regulation in 37 C.F.R 401 for leaving the enforcement of reasonable prices up to the agencies.<sup>64</sup> Finally, Arno and Davis accuse the GAO of committing the “fatal error of

58. *Id.* at 662.

59. Compare this with Arno and Davis’ opinion of NIH’s “unbelievable” complaints that price review is beyond its ability notwithstanding the “countless” cases and “host of” statutes to the contrary. See Arno & Davis, *supra* note 6, at 651-52.

60. Admiral Rickover in his testimony on Bayh-Dole never suggested a reasonable pricing requirement as a condition for allowing universities to retain title to their inventions made with government funds. Rather, he proposed to give universities and small businesses an automatic five-year exclusive license after which the invention would fall into the public domain, thereby obviating the need for march-in and recoupment. *University Hearing, supra* note 5, at 161-62.

61. S. Rep. 96-480, *supra* note 2, at 25-26.

62. S. 414, *supra* note 2, § 204 (Return of Government Investment).

63. Pub. L. 98-620 (1984) deleted this exclusive licensing restriction.

64. Arno & Davis, *supra* note 6, at 648-49.



confusing march-in rights with simple working requirements.”<sup>65</sup> Of course, all this criticism is misplaced, since there is no evidence that Congress intended there to be a reasonable pricing requirement in Bayh-Dole.

The authors submit that the interpretation taken by Arno and Davis is inconsistent with the intent of Bayh-Dole, especially since the Act was intended to promote the utilization of federally funded inventions and to minimize the costs of administering the technology transfer policies.<sup>66</sup> As pointed out by Justice Brennan, a thing may be within the letter of the law but not within the purpose of the law.<sup>67</sup> On the other hand, this would not be the case if agencies were responsible for ensuring reasonable prices for any patented invention, not just a drug, arising out of federal funding. Further, one of the stated objectives of Bayh-Dole, found in 35 U.S.C. § 200, is to “protect the public against nonuse or unreasonable use.”<sup>68</sup> It neither provides for, nor mentions, “unreasonable prices.”<sup>69</sup>

In H.R. 6933,<sup>70</sup> a companion bill to S. 414 which resulted in Bayh-Dole, there was a march-in rights provision—§ 387—which was similar in part to 35 U.S.C. § 203(1)(a). Under § 387(a)(1) of the provision, an agency could terminate the contractor’s title or exclusive rights, or require the contractor to grant licenses if the contractor had not taken and was not expected to take timely and effective action to achieve practical application in one or more fields

65. *Id.* at 676 n.273.

66. 35 U.S.C. § 200 (2000).

67. *United Steelworkers of America v. Weber*, 443 U.S. 193, 201 (1979), *citing* *Holy Trinity Church v. U.S.*, 143 U.S. 457, 459 (1892) and *discussed in* Judge Ruggero J. Aldisert, *The Brennan Legacy: The Art of Judging*, 32 LOY. L.A. L. REV. 673, 682-83 (1999).

68. Thus, an agency may march-in for reasons other than non-use of an invention. *See* S. REP. 96-480, *supra* note 2 at 30 (“The agencies will have the power to exercise march-in rights to insure that no adverse effects result from retention of patent rights by these contractors.”). As Dr. Betsy Ancker-Johnson, former Assistant Secretary of Commerce, explained, the purpose for march-in rights is to correct “should something go wrong” and if there is “any remote possibility of abuse.” *See University Hearing, supra* note 5 at 153-54. Unfortunately, no guidance was given on how to determine what is an abuse and this may refer to the other march-ins in 35 U.S.C. § 203(a)(2)-(4) (2000). On the other hand, there may be a situation where a contractor is using an invention for itself but not making the benefits of the invention available to the public at all or on reasonable terms, which could include price. This might be a basis for march-in as mentioned by David Halperin, *The Bayh-Dole Act and March-in Rights*, at 6 (May 2001) available at <http://www.essentialinventions.org/legal/norvir/halperinmarchin2001.pdf> (last visited September 1, 2005), although we disagree with the “reasonable pricing” arguments he adopted from Arno and Davis.

69. Arno & Davis, *supra* note 6 at 683, argued that “unreasonable use” includes unreasonable prices.

70. H.R. 6933, 96th Cong. (1980).

of use. According to the legislative history,<sup>71</sup> this section was “intended to continue existing practice and the [House Judiciary] Committee intends that agencies continue to use the march-in provisions in a restrained and judicious manner as in the past.”<sup>72</sup>

Although H.R. 6933 ultimately incorporated S. 414, the discussion by the House Judiciary Committee is considered relevant to 35 U.S.C. § 203 because of the similarity in language and the fact that it is included in the legislative history of Bayh-Dole. Thus, it does not appear that Congress intended that there be any change in the application of march-in rights by the agencies, which prior to that time focused on the non-utilization or non-working of federally funded patented inventions, as is evident from the previous discussion of the history under the Presidential Memoranda and the IPAs.<sup>73</sup>

The authors recognize that 35 U.S.C. § 203 includes the language “available on reasonable terms,” but one has to understand the context of the phrase in the statute. As previously mentioned with respect to the history of march-in and the two recent petitions to HHS, that term relates only to licensing. Thus, a university licensing its invention to a drug company that sells the patented product to the public is fulfilling its responsibility under Bayh-Dole of making the benefits of the invention available to the public on reasonable terms.

Although we disagree with the interpretation of 35 U.S.C. § 203 by Arno and Davis, Congress could decide to amend Bayh-Dole to impose a reasonable pricing requirement. However, we would not recommend such a change because of the difficulty in determining what is “reasonable.”<sup>74</sup> Furthermore, that would make any patent license granted by a Government contractor or grantee subject to attack,<sup>75</sup> which would discourage or inhibit the commercialization of

71. H.R. REP. NO. 96-1307, pt.1 (1980) *reprinted in* 1980 U.S.C.C.A.N. 6460, 6474.

72. *Id.* at 6474.

73. See *Koons Buick Pontiac GMC, Inc. v. Nigh*, 125 S. Ct. 460, 468 (2004), *citing* *Church of Scientology of Cal. v. IRS*, 484 U.S. 9, 17-18 (1987), where the Supreme Court focused on the lack of Congressional intent to significantly change the meaning of a clause by referring to a Sherlock Holmes story. (“All in all, we think this is a case where common sense suggests, by analogy to Arthur Conan Doyle’s ‘dog that didn’t bark . . .’”). It is remarkable that there is no discussion in the legislative history of Bayh-Dole about a reasonable pricing requirement.

74. See testimony of Dr. Bernadine Healy, Director of NIH, on Feb. 24, 1993 that NIH is not equipped, either by its expertise or its legislative mandate, to analyze private sector product pricing decisions. Arno & Davis, *supra* note 6, at 670 n.245. Such a determination would be further complicated by when it is done because of the long time and large funds it takes to get to get a drug to market.

75. Although 35 U.S.C. § 203 (2000) applies only to nonprofit organizations and small business firms, it was expanded to large businesses by 35 U.S.C. § 210(c) (2000).

Government-funded technology, one of the primary purposes of the Act.<sup>76</sup>

It is of interest that NIH had a reasonable pricing policy several years ago. In October 1991, NIH put a reasonable pricing clause in an exclusive patent license with Bristol-Myers-Squibb for the use of ddI, a new AIDS drug.<sup>77</sup> Around this time, NIH also had a reasonable pricing clause in all of its cooperative research and development agreements (CRADAs).<sup>78</sup> Dr. Harold Varmus, the Director of NIH, withdrew the reasonable pricing requirement in its CRADAs in 1995 after convening panels of scientists and administrators in government, industry, universities, and patient advocacy groups to review this policy.<sup>79</sup> In a recent report to Congress, NIH acknowledges that “[t]he cost of prescription drugs is a legitimate public concern that exists whether or not a drug was developed from a technology arising from federally funded research . . . [but NIH] has neither the mandate nor the authority to be the arbiter of drug affordability.”<sup>80</sup>

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76. This could be especially damaging for biotech inventions. See McCabe, *supra* note 31, at 648. However, a contrary view is taken by Mary Eberle, *March-In Rights Under the Bayh-Dole Act: Public Access to Federally Funded Research*, 3 MARQ. INTELL. PROP. L. REV. 155, 171 (1999) (“I argue, by contrast, that a march-in under one of the four circumstances enumerated in the Act would not harm technology transfer.”).

77. *The National Institutes of Health and its Role in Creating U.S. High-Technology Industry Growth and Jobs: Hearing before the Subcomm. on Regulation, Business Opportunities, and Energy of the House Comm. on Small Business*, 102d Cong. 9 (1991). When then Congressman Wyden asked about objections to this policy at NIH, Dr. Healy explained that “we are not interested in price setting, but we are interested in using our leverage.” *Id.* at 22. She repeated later that NIH should not be involved in price setting. *TPCC Report: Hearing before Subcomm. on Regulation, Business Opportunities, and Technology of House Comm. on Small Business*, 103rd Cong. 16 (1993).

78. Arno and Davis suggest that march-in rights apply to CRADAs although they are not funding agreements as defined by Bayh-Dole. See Arno & Davis, *supra* note 6, at 644-45. However, CRADAs have their own march-in rights provision in 15 U.S.C. § 3710a(b)(1)(B) and (C) (2000), although it is more limited than 35 U.S.C. § 203 (2000) and does not refer to “practical application.” The only mention of reasonable terms is with respect to a license to be granted by the Government in § 3710a(b)(1)(B)(i). Similarly, there is a march-in like right in the licensing of a Government-owned invention provided in 35 U.S.C. § 209(f)(2) and (4) (2000) under which the Government may terminate the license.

79. National Institutes of Health, *NIH Response to the Conference Report Request for a Plan to Ensure Taxpayers’ Interests are Protected* (July 2001), available at <http://www.nih.gov/news/070101wyden.htm> (last visited September 1, 2005).

80. National Institutes of Health, *Affordability of Inventions and Products*, at 4 (July 2004), available at [http://ott.od.nih.gov/New\\_Pages/211856ottrept.pdf](http://ott.od.nih.gov/New_Pages/211856ottrept.pdf), (last visited June 4, 2005).

## CONCLUSION

There is no reasonable pricing requirement under 35 U.S.C. § 203(1)(a)(1), considering the language of this section, the legislative history, and the prior history and practice of march-in rights. Rather, this provision is to assure that the contractor utilizes or commercializes the funded invention.<sup>81</sup>

However, that does not mean that the price charged for a drug invented with Government funding is never of concern to the funding agency. There are other mechanisms to address this concern, including the health march-in authority of 35 U.S.C. § 203(1)(a)(2), the Government license in 35 U.S.C. § 202(c)(4), and eminent domain in 28 U.S.C. § 1498(a).<sup>82</sup> In addition, NIH asserted co-inventorship in AZT, which contributed to reducing the cost for this important AIDS drug, sold by Burroughs Wellcome, even though the claim of co-ownership was not sustained in court.<sup>83</sup> Finally, discriminatory pricing of drugs, whether or not invented with Government funds, may fall within the responsibility of the Federal Trade Commission if it can be found to be anti-competitive behavior in violation of antitrust laws.<sup>84</sup>

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81. See Alstadt, *supra* note 20, at 81.

82. See McGarey & Levey, *supra* note 36, at 1113-15.

83. See Lacy et al., *Technology Transfer Laws Governing Federally Funded Research and Development*, 19 PEPP. L. REV. 1, 2 (1991), and Ackiron, *The Human Genome Initiative and the Impact of Genetic Testing and Screening Technologies: Note and Comment: Patents for Critical Pharmaceuticals: The AZT Case*, 17 AM. J.L. & MED. 145 (1991). Dr. Healy explained that the licensing of AZT by NIH was to lower Burroughs Wellcome's price, which went from \$8,000-10,000 to \$2,000. *The National Institutes of Health and its Role in Creating U.S. High-Technology Industry Growth and Jobs: Hearing before the Subcommittee on Regulation, Business Opportunities, and Energy of the House Committee on Small Business*, 102d Cong. 23 (1991).

84. See NIH decision on the Norvir® march-in petition. National Institutes of Health, *supra* note 42.

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**From:** Lambertson, David (NIH/NCI) [E] [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=3C95B34F709746A8A2553CE54E74ACE2-LAMBERTSOND]  
**Sent:** 6/11/2018 1:25:35 PM  
**To:** Rodriguez, Richard (NIH/NCI) [E] [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=8092cb5394e04733ac0d4d84d25f65e5-rodgr]; Rohrbaugh, Mark (NIH/OD) [E] [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=591ab6b2424b4b8997082718cbb29fab-rohrbaum]  
**Subject:** RE: BEORO Therapeutics license

Let's do 10:30 then. Since there are three of us, I will set up a conference number (unless either of you wants to set up the number for some reason).

David A. Lambertson, Ph.D.  
Senior Technology Transfer Manager  
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National Cancer Institute/NIH  
[david.lambertson@nih.gov](mailto:david.lambertson@nih.gov)  
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**From:** Rodriguez, Richard (NIH/NCI) [E]  
**Sent:** Monday, June 11, 2018 9:23 AM  
**To:** Lambertson, David (NIH/NCI) [E] <david.lambertson@nih.gov>; Rohrbaugh, Mark (NIH/OD) [E] <rohrbaum@od.nih.gov>  
**Subject:** RE: BEORO Therapeutics license

Both would work for me.

**From:** Lambertson, David (NIH/NCI) [E]  
**Sent:** Monday, June 11, 2018 8:30 AM  
**To:** Rohrbaugh, Mark (NIH/OD) [E] <rohrbaum@od.nih.gov>  
**Cc:** Rodriguez, Richard (NIH/NCI) [E] <richard.rodriquez@nih.gov>  
**Subject:** RE: BEORO Therapeutics license

Either time works for me. Richard, do you have a preference?

David A. Lambertson, Ph.D.  
Senior Technology Transfer Manager  
Technology Transfer Center  
National Cancer Institute/NIH  
[david.lambertson@nih.gov](mailto:david.lambertson@nih.gov)  
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**From:** Rohrbaugh, Mark (NIH/OD) [E]  
**Sent:** Monday, June 11, 2018 8:18 AM  
**To:** Lambertson, David (NIH/NCI) [E] <david.lambertson@nih.gov>  
**Cc:** Rodriguez, Richard (NIH/NCI) [E] <richard.rodriguez@nih.gov>  
**Subject:** Re: BEORO Therapeutics license

10:30 or 11?

Sent from my iPhone

On Jun 11, 2018, at 6:50 AM, Lambertson, David (NIH/NCI) [E] <david.lambertson@nih.gov> wrote:

What time would be good for a brief call for you both this morning/afternoon? Earlier would be better as I received a second e-mail from Mr. Love late Friday evening (1 day after his first e-mail), so I would like to get back to him sooner rather than later.

Thanks,

David A. Lambertson, Ph.D.  
Senior Technology Transfer Manager  
Technology Transfer Center  
National Cancer Institute/NIH  
[david.lambertson@nih.gov](mailto:david.lambertson@nih.gov)  
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Fax: 240-276-5504

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**From:** Rohrbaugh, Mark (NIH/OD) [E]  
**Sent:** Thursday, June 07, 2018 2:31 PM  
**To:** Lambertson, David (NIH/NCI) [E] <david.lambertson@nih.gov>; Rodriguez, Richard (NIH/NCI) [E] <richard.rodriguez@nih.gov>  
**Subject:** RE: BEORO Therapeutics license

REL0000024044



Let me know if you want to talk about it by phone.

**From:** Lambertson, David (NIH/NCI) [E]  
**Sent:** Thursday, June 07, 2018 2:13 PM  
**To:** Rodriguez, Richard (NIH/NCI) [E] <[richard.rodriguez@nih.gov](mailto:richard.rodriguez@nih.gov)>; Rohrbaugh, Mark (NIH/OD) [E] <[RohrBauM@OD.NIH.GOV](mailto:RohrBauM@OD.NIH.GOV)>  
**Subject:** FW: BEORO Therapeutics license

FYI, it appears that KEI intends to object to the FR Notice that published today. See e-mail below.

David A. Lambertson, Ph.D.  
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**From:** James Love [<mailto:james.love@keionline.org>]  
**Sent:** Thursday, June 07, 2018 5:00 AM  
**To:** Lambertson, David (NIH/NCI) [E] <[david.lambertson@nih.gov](mailto:david.lambertson@nih.gov)>  
**Subject:** BEORO Therapeutics license

David A. Lambertson, Ph.D.,  
Senior Technology Transfer Manager,  
NCI Technology Transfer Center  
Email:

[david.lambertson@nih.gov](mailto:david.lambertson@nih.gov).

David, we intend to file comments on this license. Below is a draft of what we will file, probably with other groups. We would like to call to explain the motivation for these various requests, including the transparency proposals.

Also, can you answer a few questions about the license.

1. What is the proposed consideration for the exclusive license?
2. Are there any former NIH employees involved with the company?
3. Does this company have a track record of developing new drugs or treatments?
4. Will the company manufacture or conduct research in the United States.

5. Did the NIH do any analysis to see if a term that is less than the life of a patent would be appropriate and sufficient?
6. Did the NIH ask DOJ for a review, under 40 USC 559?

Jamie

Knowledge Ecology International (KEI), , and \_\_\_\_\_ are organizations concerned about drug pricing and access to patented medicines, offering comments on the grant of an exclusive license, between the National Institutes of Health (NIH) and

BEORO Therapeutics, GmbH. ("Beoro") located in Seefeld, Germany, for patents noticed in the Federal Register (83 FR 26487) the Development of an Anti-BCMA Immunotoxin for the Treatment of Human Cancer.

(See: <https://www.gpo.gov/fdsys/pkg/FR-2018-06-07/pdf/2018-12179.pdf>)

The above entities oppose the issuing of the license unless:

A. The NIH has determined that an exclusive license is "a reasonable and necessary incentive" to induce investments for the development and practical application of the invention, as is required by 35 USC § 209, and shares its analysis with the public; and

B. The NIH limits the scope of rights for the exclusivity to only those rights reasonably necessary to induce investments for the development and practical application of the invention, and in particular, that the field of use is sufficiently narrow, that the term of the exclusivity is sufficiently limited, and that the license contains sufficient safeguards to ensure that the invention is "available to the public on reasonable terms," as is required by 35 USC § 209 and 35 USC § 201(f).

Our comments address three areas of concern, (1) the pricing, affordability and access issues, (2) freedom for researchers to use the inventions, and (3) requirements for transparency of the development and commercialization of the medicine.

We propose the following safeguards regarding the pricing of and access to products that use the inventions:

1.

Products are priced no higher in the United States than the median price charged in the seven largest economies as measured by nominal GNI that have a nominal GNI per capita of at least 50 percent of the United States. To fully appreciate our concerns about the discriminatory pricing that makes US residents pay more than everyone else, please review the cross country price comparisons here: <http://drugdatabase.info/drug-prices/>

2.

Prices for products in the United States do not exceed the estimated value of the treatment, as determined by independent health technology assessments selected by Department of Health and Human Services (HHS).

3.

Patient co-payments under third party Medicare and private reimbursement programs are affordable.

4.

The geographic area for the exclusivity should exclude countries with a per capita income less than 30 percent that of the United States. If there is no such exclusion, the company be required to report annually on the reasonable and feasible measures that will be taken to ensure access to patients living in such countries. Here, please note the data from <http://drugdatabase.info/drug-prices/>, which shows that in many developing countries, prices are frequently higher than the prices for high income countries in Europe, despite the much lower per capita income in developing countries (including for taxpayer funded cancer drugs), illustrating the need for a policy to be included in NIH licenses. We also note the Medicines Patent Pool (MPP) has recently announced it will expand the scope of diseases for its licenses. The NIH should retain the flexibility to provide licenses to the MPP in the future, perhaps as an option clause in the license.

5.

The initial period of exclusivity is set at seven years, subject to extensions if the company can demonstrate it has not recovered sufficient profits given the risk-adjusted value of the clinical trials used to register similar drugs for the lead indication.

6.

Absent satisfaction of the requirements of proposed safeguard number 5, the exclusivity of the product be reduced when cumulative global revenues for the product exceed \$1 billion, by one year for every \$0.5 billion in cumulative sales that exceed \$1 billion in cumulative sales.

The NIH might consider a different set of benchmarks than \$1 billion and \$.5 billion. In considering any benchmarks for global sales benchmarks, note that the licensing of inventions to the company significantly reduces the company's costs of preclinical research, which various studies have estimated to be 40 to 55 percent of drug development costs on a risk- and capital cost-adjusted basis.

To address research by third parties on the patented invention, we propose the NIH explicitly permit researchers worldwide to use the inventions for research purposes, regardless of whether or not research has a grant or contract from a U.S. government agency, and for both profit or non-profit organizations.

To address transparency, we proposes the company be required to provide an annual report for the public providing disclosures of the following items:

1.

The amount of money R&D to obtain FDA and foreign government approvals of the inventions, including in particular, the amount of money spent each year on each trial, and the relevant tax credits, grants and other subsidies received from any government or charity relating to those R&D outlays,

2.

The prices and revenue for the products, by country,

3.

The number of units sold, in each country,

4.

The product-relevant patents obtained in each country, and

5.

The regulatory approval obtained in each country.

--

James Love. Knowledge Ecology International

<http://www.keionline.org/donate.html>

KEI DC tel: +1.202.332.2670, US Mobile: +1.202.361.3040, Geneva Mobile: +41.76.413.6584,

[twitter.com/jamie\\_love](https://twitter.com/jamie_love)

Thanks Mark. Claire, you can follow up.

On Thu, Jan 12, 2017 at 12:35 PM, Rohrbaugh, Mark (NIH/OD) [E] <[RohrBauM@od.nih.gov](mailto:RohrBauM@od.nih.gov)> wrote:

Jamie:

I can talk to her and am available today after 3:30 or tomorrow 10-11 or 2-4

Mark

**From:** jamespackardlove@gmail.com [mailto:jamespackardlove@gmail.com] **On Behalf Of** Jamie Love  
**Sent:** Wednesday, January 11, 2017 2:08 PM  
**To:** Rohrbaugh, Mark (NIH/OD) [E] <RohrBauM@OD.NIH.GOV>  
**Cc:** Claire Cassedy <claire.cassedy@keionline.org>  
**Subject:** For Mark

Mark, is there someone at the NIH that Claire can talk to about the policy on publishing notices about CRADAs?

We were surprised at few notices we found in the Federal Register.

Jamie

—

James Love. Knowledge Ecology International  
<http://www.keionline.org/donate.html>

REL0000024047

KEI DC tel: +1.202.332.2670, US Mobile: +1.202.361.3040, Geneva Mobile: +41.76.413.6584,  
twitter.com/jamie\_love

--

James Love. Knowledge Ecology International  
<http://www.keionline.org/donate.html>

KEI DC tel: +1.202.332.2670, US Mobile: +1.202.361.3040, Geneva Mobile: +41.76.413.6584,  
twitter.com/jamie\_love

---

**From:** Joe Allen [jallen@allen-assoc.com]  
**Sent:** 4/15/2019 9:34:22 PM  
**To:** Rohrbaugh, Mark (NIH/OD) [E] [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=591ab6b2424b4b8997082718cbb29fab-rohrbaum]; Hammersla, Ann (NIH/OD) [E] [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=87fb28aa23744c0b855ef0683ac2e8b4-hammerslaa]  
**Subject:** Judge dismisses KEI suit against NIH exclusive license

Score one for the good guys (<https://www.keionline.org/30396>)

## Litigation: KEI lawsuit over NIH/Gilead CAR T License Dismissed over Lack of Standing

Posted on April 12, 2019 by KEI Staff

In an order dated April 11, 2019, Judge Peter J. Messitte granted the NIH's motion to dismiss for lack of jurisdiction in the lawsuit filed by KEI, which appealed an NIH decision to grant an exclusive license on a CAR T cancer therapy to Gilead. During the licensing process, the NIH refused to disclose the amount of money it was spending on a 77 patient clinical trial, or to include any provisions protecting U.S. residents from prices higher than the median of other high income countries, or to include measures to ensure access in developing countries, or to consider just delaying a license until the NIH had results from its own government-funded clinical trial.

The Judge did not resolve a dispute over whether or not the Bayh-Dole Act somehow eliminates obligations in 40 U.S.C. § 599, as the NIH alleges.

The memorandum opinion is [available here](#). The final order is [available here](#).

*(More pleadings on the case here: <https://www.keionline.org/kei-v-nih>)*

Judge Messitte found that while "KEI is an admirable organization that undoubtedly possesses expertise in the issues going to the merits of this case," KEI lacked organizational and associational standing to sue the NIH over the licensing decision.

### Comment by James Love, KEI Director

Judge Messitte's decision illustrates how little influence citizens have over the licensing practices of the NIH, despite the putative opportunity to comment on proposed exclusivity licenses, and the policy and objective of the Bayh-Dole Act to "protect the public against nonuse or unreasonable use of inventions." [35 USC § 200]. While the decision leaves the door open to challenge NIH licensing practices for a different license with different plaintiffs that can demonstrate the requisite harm, the NIH will likely become even more emboldened to ignore criticisms of its lack of transparency and failure to provide provisions to protect the public from excessive and abusive prices. At some point, Congress needs to provide real oversight of the NIH management of the public's rights in federally-funded inventions.

--  
Joseph P. Allen  
President  
Allen and Associates  
60704 Rt. 26, South

REL0000024048

Bethesda, OH 43719

(W) 740-484-1814

(c) b6

[www.allen-assoc.com](http://www.allen-assoc.com)



---

**From:** Koniges, Ursula (NIH/OD) [E] [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=D5AE2C3139654BC0B9B95718D516310B-KONIGESUM]  
**Sent:** 1/19/2018 7:48:52 PM  
**To:** Rohrbaugh, Mark (NIH/OD) [E] [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=591ab6b2424b4b8997082718cbb29fab-rohrbaum]  
**CC:** Dodson, Sara (NIH/OD) [E] [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=985a956eaa0d4945bdcfd8ea30947d68-dodsonse]  
**Subject:** RE: Here is the BRAIN rough draft  
**Attachments:** BRAIN Drug Pricing MR Draft - v4.docx

Hi Mark,

Quick heads-up that Sara and I will touch base with you this afternoon about the Drug Pricing BRAIN brief. I've attached a slightly updated v4.docx with a minor edit.

Thanks,  
-Ursula

**From:** Koniges, Ursula (NIH/OD) [E]  
**Sent:** Thursday, January 18, 2018 2:12 PM  
**To:** Dodson, Sara (NIH/OD) [E] <sara.dodson@nih.gov>  
**Cc:** Rohrbaugh, Mark (NIH/OD) [E] <RohrBauM@OD.NIH.GOV>  
**Subject:** RE: Here is the BRAIN rough draft

Slightly updated v3.docx attached with a reference added for the article Mark just sent.

**From:** Koniges, Ursula (NIH/OD) [E]  
**Sent:** Thursday, January 18, 2018 2:03 PM  
**To:** Dodson, Sara (NIH/OD) [E] <sara.dodson@nih.gov>  
**Cc:** Rohrbaugh, Mark (NIH/OD) [E] <RohrBauM@OD.NIH.GOV>  
**Subject:** RE: Here is the BRAIN rough draft

Hi Sara,

Mark shared a draft of the Drug Pricing BRAIN brief, and mentioned that it would be useful for us to look over it (for organization, minor editing, etc.) before it's uploaded onto BRAIN. I've attached Mark's original .docx (with his edits in track changes), and v2 (with my edits) that we can take from here.

Safe trip, and see you tomorrow!  
-Ursula

**From:** Rohrbaugh, Mark (NIH/OD) [E]  
**Sent:** Tuesday, January 16, 2018 6:17 PM  
**To:** Koniges, Ursula (NIH/OD) [E] <ursula.koniges@nih.gov>  
**Subject:** Here is the BRAIN rough draft

Thx

Mark L. Rohrbaugh, Ph.D., J.D.  
Special Advisor for Technology Transfer

REL0000024052

Director, Division of Technology Transfer and Innovation Policy  
Office of Science Policy  
Office of the Director  
National Institutes of Health

**b5**

**b5**

**b5**

---

**From:** Myles, Renate (NIH/OD) [E] [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=7D317F5626934585B3692A1823C1B522-MYLESR]  
**Sent:** 6/27/2018 7:42:48 PM  
**To:** Rohrbaugh, Mark (NIH/OD) [E] [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=591ab6b2424b4b8997082718cbb29fab-rohrbaum]  
**CC:** Burklow, John (NIH/OD) [E] [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=2e57f267323b43c08be856acb5b964ca-burklowj]; Fine, Amanda (NIH/OD) [E] [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=61290b74aa9a44358954c45439ffdeb6-fineab]; Wojtowicz, Emma (NIH/OD) [E] [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=45c6610aca6e44a08d497630425e5ecd-wojtowiczem]  
**Subject:** Comms Plan on NIH inventions  
**Attachments:** Draft\_Communication\_Plan\_NIH\_Inventions.6.21.18.docx

Hi Mark:

Here is a first draft of the communication plan on NIH inventions. Sorry it's taken so long to get this to you. We never actually have down time in my branch. Key messages and an actual schedule still need to be drafted, but I wanted to put this into motion since I will be out of town beginning tomorrow through the July 9. However, Amanda and Emma can keep it moving. I just wanted to make sure I captured the issue accurately.

Thanks,  
Renate

**b5**



**b5**

**b5**

**From:** Hammersla, Ann (NIH/OD) [E] [/O=NIH/OU=NIHEXCHANGE/CN=RECIPIENTS/CN=HAMMERSLAA]  
**Sent:** 11/3/2016 5:27:11 PM  
**To:** Rohrbaugh, Mark (NIH/OD) [E] [/O=NIH/OU=NIHEXCHANGE/cn=OD/cn=ROHRBAUM]  
**Subject:** RE: 2 things

Hi Mark:

**b5**

**b5**

I am available now to talk about TTPB or next Mon-Wednesday.

Ann

**From:** Rohrbaugh, Mark (NIH/OD) [E]  
**Sent:** Wednesday, November 02, 2016 5:20 PM  
**To:** Hammersla, Ann (NIH/OD) [E] <hammerslaa@mail.nih.gov>  
**Subject:** 2 things

**b5**

THanks,  
Mark

Mark L. Rohrbaugh, Ph.D., J.D.  
Special Advisor for Technology Transfer  
Director, Division of Technology Transfer and Innovation Policy  
Office of Science Policy  
National Institutes of Health

---

**From:** Berkley, Dale (NIH/OD) [E] [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=5EE461C29F5045A49F0ADF82CAAA2F31-BERKLEYD]  
**Sent:** 7/16/2019 6:56:44 PM  
**To:** Rohrbaugh, Mark (NIH/OD) [E] [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=591ab6b2424b4b8997082718cbb29fab-rohrbaum]  
**Subject:** RE: The Kite CAR CD19 and CD 20 license

Thanks

**From:** Rohrbaugh, Mark (NIH/OD) [E] <rohrbaum@od.nih.gov>  
**Sent:** Tuesday, July 16, 2019 1:53 PM  
**To:** Berkley, Dale (NIH/OD) [E] <berkleyd@od.nih.gov>  
**Subject:** FW: The Kite CAR CD19 and CD 20 license

FYI, in case this brews up

**From:** Rohrbaugh, Mark (NIH/OD) [E]  
**Sent:** Tuesday, July 16, 2019 1:53 PM  
**To:** Lambertson, David (NIH/NCI) [E] <david.lambertson@nih.gov>  
**Subject:** RE: The Kite CAR CD19 and CD 20 license

I agree

**From:** Lambertson, David (NIH/NCI) [E] <david.lambertson@nih.gov>  
**Sent:** Tuesday, July 16, 2019 1:52 PM  
**To:** Rohrbaugh, Mark (NIH/OD) [E] <rohrbaum@od.nih.gov>  
**Subject:** RE: The Kite CAR CD19 and CD 20 license

Thanks.

b5

**b5**

David A. Lambertson, Ph.D.  
Senior Technology Transfer Manager  
Technology Transfer Center  
National Cancer Institute/NIH  
[david.lambertson@nih.gov](mailto:david.lambertson@nih.gov)  
<http://ttc.nci.nih.gov/>

9609 Medical Center Drive, Rm 1-E530 MSC 9702  
Bethesda, MD 20892-9702 (USPS)  
Rockville, MD 20850-9702 (Overnight/express mail)  
Phone (Main Office): 240-276-5530  
Phone (direct): (240) 276-6467  
Fax: 240-276-5504

REL0000024058

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**From:** Rohrbaugh, Mark (NIH/OD) [E]  
**Sent:** Tuesday, July 16, 2019 1:15 PM  
**To:** Lambertson, David (NIH/NCI) [E] <david.lambertson@nih.gov>  
**Subject:** RE: The Kite CAR CD19 and CD 20 license

I would say

b5

b5

**From:** Lambertson, David (NIH/NCI) [E] <david.lambertson@nih.gov>  
**Sent:** Tuesday, July 16, 2019 1:13 PM  
**To:** Rohrbaugh, Mark (NIH/OD) [E] <rohrbaum@od.nih.gov>  
**Subject:** FW: The Kite CAR CD19 and CD 20 license

Hi Mark,

I also just received James Love's further response to my response to Clair Cassedy, sent this morning.

b5

b5

Thanks,  
Dave

David A. Lambertson, Ph.D.  
Senior Technology Transfer Manager  
Technology Transfer Center  
National Cancer Institute/NIH  
[david.lambertson@nih.gov](mailto:david.lambertson@nih.gov)  
<http://ttc.nci.nih.gov/>

9609 Medical Center Drive, Rm 1-E530 MSC 9702  
Bethesda, MD 20892-9702 (USPS)  
Rockville, MD 20850-9702 (Overnight/express mail)  
Phone (Main Office): 240-276-5530  
Phone (direct): (240) 276-6467  
Fax: 240-276-5504

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**From:** James Love <james.love@keionline.org>  
**Sent:** Tuesday, July 16, 2019 12:20 PM  
**To:** Lambertson, David (NIH/NCI) [E] <david.lambertson@nih.gov>  
**Cc:** Claire Cassedy <claire.cassedy@keionline.org>; Kathryn Ardizzone <kathryn.ardizzone@keionline.org>; Luis Gil Abinader <luis.gil.abinader@keionline.org>; Manon Ress <MANON.RESS@cancerunion.org>  
**Subject:** The Kite CAR CD19 and CD 20 license

David A. Lambertson, Ph.D.

REL0000024058

Senior Technology Transfer Manager

Technology Transfer Center  
National Cancer Institute/NIH  
[david.lambertson@nih.gov](mailto:david.lambertson@nih.gov)

<http://ttc.nci.nih.gov/>

Phone (direct): (240) 276-6467

Dear Dr. Lambertson,

In failing to address Claire Cassedy's questions 1-5, from her July 12 email, you state: "The other questions either have been answered previously or are not related to the criteria set forth in 37 CFR 404.7(a)(1)(ii-iii) regarding a decision by a federal agency to grant an exclusive license."

Her 1-4 questions were:

1. At what stage of development are the inventions listed?
2. Has the government funded any clinical trials relevant to these technologies?
3. If the government has provided funding, how much has been spent by the government on these trials?  
Can you provide NCT numbers?
4. Is the term in the proposed licenses to be life of patent or less than life of patent?

Her question 5 was:

5. In working towards executing this license, has the NIH sought advice from the Attorney General (as is required under 40 USC § 559) to determine if the "disposal to a private interest would tend to create or maintain a situation inconsistent with antitrust law"?

37 CFR 404.7(a)(1)(ii) states:

- (ii) After expiration of the period in §404.7(a)(1)(i) and consideration of any written objections received during the period, the Federal agency has determined that;
- (A) The interests of the Federal Government and the public will best be served by the proposed license, in view of the applicant's intentions, plans, and ability to bring the invention to practical application or otherwise promote the invention's utilization by the public;
- (B) The desired practical application has not been achieved, or is not likely expeditiously to be achieved, under any nonexclusive license which has been granted, or which may be granted, on the invention;
- (C) Exclusive or partially exclusive licensing is a reasonable and necessary incentive to call forth the investment of risk capital and expenditures to bring the invention to practical application or otherwise promote the invention's utilization by the public; and
- (D) The proposed terms and scope of exclusivity are not greater than reasonably necessary to provide the incentive for bringing the invention to practical application or otherwise promote the invention's utilization by the public;

(C) and (D) of (ii) go to issues that are directly related to questions 1-4. Obviously, if a company is licensing technologies in very early stages of development where no trials have been funded or subsidized, they will need more robust incentives than when a technology is already further along, and the government is funding trials. Failing to respond to Claire's questions is withholding information that we need to comment on the proposed license.

As regards Claire's question 5, regarding advice from the Attorney General, this is directly relating to due diligence for 37 CFR 404.7(a)(1)(iii), which states:

(iii) The Federal agency has not determined that the grant of such license will tend substantially to lessen competition or result in undue concentration in any section of the country in any line of commerce to which the technology to be licensed relates, or to create or maintain other situations inconsistent with the antitrust laws; and

--

James Love. Knowledge Ecology International  
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U.S. office phone +1.202.332.2670  
<http://www.keionline.org>  
[twitter.com/jamie\\_love](https://twitter.com/jamie_love)



---

**From:** Berkley, Dale (NIH/OD) [E] [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=5EE461C29F5045A49F0ADF82CAAA2F31-BERKLEYD]  
**Sent:** 1/2/2019 7:37:23 PM  
**To:** Rohrbaugh, Mark (NIH/OD) [E] [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=591ab6b2424b4b8997082718cbb29fab-rohrbaum]  
**Subject:** RE: Question from Alan [b5]

b5

**From:** Rohrbaugh, Mark (NIH/OD) [E] <rohrbaum@od.nih.gov>  
**Sent:** Wednesday, January 02, 2019 2:25 PM  
**To:** Berkley, Dale (NIH/OD) [E] <berkleyd@od.nih.gov>  
**Subject:** RE: Question from Alan [b5]

Yes. [b5]

**From:** Berkley, Dale (NIH/OD) [E] <berkleyd@od.nih.gov>  
**Sent:** Wednesday, January 02, 2019 2:07 PM  
**To:** Rohrbaugh, Mark (NIH/OD) [E] <rohrbaum@od.nih.gov>  
**Subject:** RE: Question from Alan [b5]

b5

**From:** Rohrbaugh, Mark (NIH/OD) [E] <rohrbaum@od.nih.gov>  
**Sent:** Wednesday, January 02, 2019 11:42 AM  
**To:** Berkley, Dale (NIH/OD) [E] <berkleyd@od.nih.gov>  
**Subject:** RE: Question from Alan [b5]

b5

**From:** Berkley, Dale (NIH/OD) [E] <berkleyd@od.nih.gov>  
**Sent:** Wednesday, January 02, 2019 9:48 AM  
**To:** Rohrbaugh, Mark (NIH/OD) [E] <rohrbaum@od.nih.gov>  
**Subject:** Question from Alan [b5]

Mark:

**b5**

Thanks, Dale

Dale D. Berkley, Ph.D., J.D.  
Office of the General Counsel, PHD, NIH Branch  
Bldg. 31, Rm. 47  
Bethesda, MD 20892  
301-496-6043  
301-402-2528(Fax)

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REL0000024061

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**From:** Kassilke, Deborah (NIH/OD) [E] [/O=NIH/OU=NIEXCHANGE/CN=OD/CN=KASSILKE]  
**Sent:** 1/12/2017 5:06:29 PM  
**To:** Rohrbaugh, Mark (NIH/OD) [E] [/O=NIH/OU=NIEXCHANGE/cn=OD/cn=ROHRBAUM]; Rogers, Karen (NIH/OD) [E] [/O=NIH/OU=NIEXCHANGE/cn=OD/cn=GarrettK]  
**Subject:** Re:  
**Attachments:** KEI LOVE Qs 011217.docx

Hi Mark –

In the past 10 or so business days we have received 3 requests from KEI/LOVE. I've captured them in the attached word doc.

**b5**

I will find out who in FOIA to work with given Susan C is gone, but would like your thoughts.  
Deb

*Deborah Kassilke  
Director, Office of Technology Transfer  
National Institutes of Health  
6011 Executive Boulevard, Suite 325  
Rockville, MD 20852  
E-Mail: [Deborah.Kassilke@nih.gov](mailto:Deborah.Kassilke@nih.gov)  
Phone: 301-435-5294  
Cell: **b6***

#1

**From:** jamespackardlove@gmail.com [mailto:jamespackardlove@gmail.com] **On Behalf Of** Jamie Love  
**Sent:** Friday, January 06, 2017 3:48 PM  
**To:** Kassilke, Deborah (NIH/OD) [E] <deborah.kassilke@nih.gov>  
**Cc:** Manon Ress <manon.ress@keionline.org>  
**Subject:** CRADA agreements

Dear Director Kassilke

KEI is trying to evaluate the NIH CRADA program (and similar programs in other agencies). We used FederalRegister.Gov to find a list of NIH CRADA agreements, but only found 171 public notices since 1995. We know the number of CRADAs is much larger than this. Is there some master registry or list that we can obtain?

James Love  
Director  
Knowledge Ecology International

--

James Love. Knowledge Ecology International  
<http://www.keionline.org/donate.html>  
KEI DC tel: +1.202.332.2670, US Mobile: +1.202.361.3040, Geneva Mobile: +41.76.413.6584,  
[twitter.com/jamie\\_love](https://twitter.com/jamie_love)

#2

**From:** Hammersla, Ann (NIH/OD) [E]  
**Sent:** Wednesday, January 11, 2017 1:51 PM  
**To:** Claire Cassedy <claire.cassedy@keionline.org>  
**Cc:** Goldstein, Bruce (NIH/OD) [E] <goldsteb@mail.nih.gov>  
**Subject:** RE: Request for information about CRADA process

Dear Claire:

Thank you for your email. I will not be able to provide you with answers to your CRADA questions. I have copied Bruce Goldstein on this email who will be able to answer your questions or better direct you to someone who can.

Ann

--

Ann M. Hammersla, J.D.

REL0000024062.0001

Director  
Division of Extramural Inventions and Technology Resources  
Office of Policy for Extramural Research Administration  
Rockledge 1, Suite 310  
6705 Rockledge Drive  
Bethesda, Maryland 20892-7974  
PHONE: 301-435-0745

**From:** Claire Cassedy [mailto:claire.cassedy@keionline.org]  
**Sent:** Wednesday, January 11, 2017 1:36 PM  
**To:** Hammersla, Ann (NIH/OD) [E] <hammerslaa@mail.nih.gov>  
**Subject:** Request for information about CRADA process

Dear Ms. Hammersla,

I am writing to find out more information about the process by which the NIH enters into a CRADA with an industry collaborator, and was not sure where best to direct my inquiry in the NIH OTT.

Specifically, I am interested in learning about the CRADA solicitation process, transitions to licensing of technologies, and public disclosures/comment periods of those processes. There are postings soliciting partners for CRADAs on specific technologies, but I was wondering what information is available regarding what companies receive the CRADAs and how many (and which) CRADAs result in market approved drugs down the line. Would it be possible to have a brief phone call to discuss these topics? Or if you are not the appropriate contact, could you direct me to the proper person?

Thank you in advance for your time and consideration on these issues.

Best Regards,  
Claire Cassedy

--

Knowledge Ecology International (KEI)  
1621 Connecticut Avenue NW  
Suite 500  
Washington, DC 20009  
www.keionline.org  
Tel.: (202)332-2670 ext. 11  
Fax.: (202)332-2673

#3

**From:** jamespackardlove@gmail.com [mailto:jamespackardlove@gmail.com] **On Behalf Of** Jamie Love  
**Sent:** Friday, January 06, 2017 4:05 PM  
**To:** Schwetz, Brian (NIH/OD) [E] <schwetzbr@od.nih.gov>  
**Subject:** Royalties

Dear Brian Schwetz,

KEI would like to have information on the royalties paid to federal employees. Is there a particular document that lists this year by year?

James Love  
Director  
Knowledge Ecology International

--

James Love. Knowledge Ecology International  
<http://www.keionline.org/donate.html>  
KEI DC tel: +1.202.332.2670, US Mobile: +1.202.361.3040, Geneva Mobile: +41.76.413.6584,  
[twitter.com/jamie\\_love](https://twitter.com/jamie_love)

---

**From:** Joe Allen [jallen@allen-assoc.com]  
**Sent:** 4/6/2018 3:26:35 PM  
**To:** Hammersla, Ann (NIH/OD) [E] [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=87fb28aa23744c0b855ef0683ac2e8b4-hammerslaa]; Rohrbaugh, Mark (NIH/OD) [E] [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=591ab6b2424b4b8997082718cbb29fab-rohrbaum]  
**Subject:** Re: Advocacy groups urge HHS to seize Sarepta patent under Bayh-Dole

Well the good news is that with Jamie around you'll never be out of work. Despite that, enjoy the weekend, perhaps Spring will show up sometime soon...

On 4/6/2018 11:25 AM, Hammersla, Ann (NIH/OD) [E] wrote:

We are working on the request.....

**From:** Joe Allen <jallen@allen-assoc.com>  
**Sent:** Friday, April 06, 2018 11:20 AM  
**To:** Rohrbaugh, Mark (NIH/OD) [E] <RohrBauM@OD.NIH.GOV>; Hammersla, Ann (NIH/OD) [E] <hammerslaa@mail.nih.gov>  
**Subject:** Advocacy groups urge HHS to seize Sarepta patent under Bayh-Dole

Here's the latest attack from today's Endpoint News:

### **Should Sarepta's patents be seized by the government? Patient advocates pitch controversial drug pricing proposal**

Six advocacy groups are sending a big ask to the federal government to lower the price of one rather expensive drug for Duchenne muscular dystrophy, petitioning health regulators to flex power it's never exercised before.

Amidst a years-long debate over drug pricing, Sarepta has hit a sensitive chord with the high price tag of its DMD drug Exondys 51 (eteplirsen), which goes for a around \$300,000 per year.

The organizations drafted a letter to the Department of Health and Human Services (HHS), pleading that Secretary Alex Azar act to lower the price of the drug. Specifically, the group wants HHS to leverage a piece of legislation called the Bayh-Dole Act — along with contractual agreements with funding agencies — to take over ownership of five patents on Exondys 51. They can do that, the group insists, because the intellectual property was backed by federal research dollars. Grant recipients are required to disclose federal funding that contributes to a patented invention on their patent application — a step that both Sarepta and the University of Western Australia failed to do.

By taking title to the patents, the HHS could leverage their position to lower the price of Exondys, the organizations said.

Analysts at Leerink, who cover Sarepta's stock \$SRPT, sent a note to investors this morning noting the unlikelihood that such action would be taken by the government.

“Bottom Line: Today's letter from several groups delivered to HHS Secretary (Alex) Azar



highlights the lengths that some are willing to go in order to force drug prices lower; however we believe these groups have an uphill battle, and even if they were to prevail there would be limited read through to other rare disease companies whose business models rely on premium pricing.”

Leerink reminds investors that a similar strategy was used against Gilead, Vertex, and Novartis, among others. Those efforts failed.

The letter writers acknowledge the action they’re requesting is unprecedented:

In the past, the federal government has, on several occasions, asked recipients of federal grants and contracts to correct failures to disclose federal funding of the inventions, but has not exercised its rights to take the title of such patents for purposes of influencing drug prices. In this respect, we recognize that we are asking HHS to do something new.

And later, the letters sound rather hopeless:

We respectfully ask for a meeting with your staff to further discuss this issue, noting that as a practical matter, if the decisions are delegated solely to the NIH (Office of Technology Transfer) staff it is highly unlikely any action will be taken to moderate the price of this drug.

Read the full letter here, written and submitted by KEI, Health GAP, Patients for Affordable Drugs, People of Faith for Access to Medicines, Social Security Works and Universities Allied for Essential Medicines.

--

Joseph P. Allen  
President  
Allen and Associates  
60704 Rt. 26, South  
Bethesda, OH 43719  
(W) 740-484-1814  
(c) b6  
[www.allen-assoc.com](http://www.allen-assoc.com)

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(c) b6  
[www.allen-assoc.com](http://www.allen-assoc.com)

**From:** NIH FOIA [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=E734B867D58F45E792D9FA7096AA146D-NIHFOIA]  
**Sent:** 9/1/2017 2:09:51 PM  
**To:** Rohrbaugh, Mark (NIH/OD) [E] [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=591ab6b2424b4b8997082718cbb29fab-rohrbaum]; Shmilovich, Michael (NIH/NHLBI) [E] [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=7dfe19bfd1d443ceb700b9f22d159a90-shmilovm]; Bordine, Roger (NIH/OD) [E] [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=a44282b444584690bbbe471966f54f1f-bordinerw]; Deutch, Alan (NIH/NHLBI) [E] [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=244d755700584812af36b5e787285647-deutcha]  
**CC:** Thomas, Gina (NIH/OD) [E] [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=7a7d21227e5643548f0a7c256b54f83f-gthomas]; Rogers, Karen (NIH/OD) [E] [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=b23ef4ca2fa14a6eb174ee611953a396-rogersk]; Burklow, John (NIH/OD) [E] [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=2e57f267323b43c08be856acb5b964ca-burklowj]  
**Subject:** RE: KEI Request FOIA Request Re: CRADAs Executed 2010-2017

Good Morning Everyone,

**b5**

Thank you.

**Roger Bordine**

Program Assistant  
Freedom of Information Office  
National Institutes of Health  
Building 31, Room 5B35  
31 Center Drive  
Bethesda, MD 20892

Phone: 301-496-5633  
Fax: 301-402-4541  
[Roger.bordine@nih.gov](mailto:Roger.bordine@nih.gov)



**From:** NIH FOIA

**Sent:** Monday, August 28, 2017 4:24 PM

**To:** Rohrbaugh, Mark (NIH/OD) [E] <rohrbaum@od.nih.gov>; Shmilovich, Michael (NIH/NHLBI) [E] <michael.shmilovich@nih.gov>; Bordine, Roger (NIH/OD) [E] <roger.bordine@nih.gov>; Deutch, Alan (NIH/NHLBI) [E] <deutcha@nhlbi.nih.gov>

**Cc:** Thomas, Gina (NIH/OD) [E] <gthomas@od.nih.gov>; Rogers, Karen (NIH/OD) [E] <rogersk@od.nih.gov>; Burklow, John (NIH/OD) [E] <burklowj@od.nih.gov>; NIH FOIA <nihfoia@od.nih.gov>

**Subject:** RE: KEI Request FOIA Request Re: CRADAs Executed 2010-2017

REL0000024065

Good afternoon everyone,

After our conference call today,

b5

b5

So please feel free to add or edit out anything in the draft email above.

b5

b5

Thanks everyone.

**Roger Bordine**

Program Assistant  
Freedom of Information Office  
National Institutes of Health  
Building 31, Room 5B35  
31 Center Drive  
Bethesda, MD 20892

Phone: 301-496-5633

Fax: 301-402-4541

[Roger.bordine@nih.gov](mailto:Roger.bordine@nih.gov)



**National Institutes of Health**  
*Turning Discovery into Health*

REL0000024065

**From:** Rohrbaugh, Mark (NIH/OD) [E]  
**Sent:** Monday, August 28, 2017 3:10 PM  
**To:** Shmilovich, Michael (NIH/NHLBI) [E] <[michael.shmilovich@nih.gov](mailto:michael.shmilovich@nih.gov)>; NIH FOIA <[nihfoia@od.nih.gov](mailto:.nihfoia@od.nih.gov)>; Bordine, Roger (NIH/OD) [E] <[roger.bordine@nih.gov](mailto:roger.bordine@nih.gov)>; Deutch, Alan (NIH/NHLBI) [E] <[deutcha@nhlbi.nih.gov](mailto:deutcha@nhlbi.nih.gov)>  
**Cc:** Thomas, Gina (NIH/OD) [E] <[gthomas@od.nih.gov](mailto:gthomas@od.nih.gov)>; Rogers, Karen (NIH/OD) [E] <[rogersk@od.nih.gov](mailto:rogersk@od.nih.gov)>  
**Subject:** RE: KEI Request FOIA Request Re: CRADAs Executed 2010-2017

Ok by me

---

**From:** Shmilovich, Michael (NIH/NHLBI) [E]  
**Sent:** Monday, August 28, 2017 2:56 PM  
**To:** Rohrbaugh, Mark (NIH/OD) [E] <[rohrbaum@od.nih.gov](mailto:rohrbaum@od.nih.gov)>; NIH FOIA <[nihfoia@od.nih.gov](mailto:.nihfoia@od.nih.gov)>; Bordine, Roger (NIH/OD) [E] <[roger.bordine@nih.gov](mailto:roger.bordine@nih.gov)>; Deutch, Alan (NIH/NHLBI) [E] <[deutcha@nhlbi.nih.gov](mailto:deutcha@nhlbi.nih.gov)>  
**Cc:** Thomas, Gina (NIH/OD) [E] <[gthomas@od.nih.gov](mailto:gthomas@od.nih.gov)>  
**Subject:** RE: KEI Request FOIA Request Re: CRADAs Executed 2010-2017

Ok I have a telecon at 15:05 can we gab at 15:30?

**From:** Rohrbaugh, Mark (NIH/OD) [E]  
**Sent:** Monday, August 28, 2017 14:56  
**To:** NIH FOIA <[nihfoia@od.nih.gov](mailto:.nihfoia@od.nih.gov)>; Shmilovich, Michael (NIH/NHLBI) [E] <[michael.shmilovich@nih.gov](mailto:michael.shmilovich@nih.gov)>; Bordine, Roger (NIH/OD) [E] <[roger.bordine@nih.gov](mailto:roger.bordine@nih.gov)>; Deutch, Alan (NIH/NHLBI) [E] <[deutcha@nhlbi.nih.gov](mailto:deutcha@nhlbi.nih.gov)>  
**Cc:** Thomas, Gina (NIH/OD) [E] <[gthomas@od.nih.gov](mailto:gthomas@od.nih.gov)>  
**Subject:** RE: KEI Request FOIA Request Re: CRADAs Executed 2010-2017

I am available the rest of today and tomorrow until 1 pm.

Mark

**From:** NIH FOIA  
**Sent:** Monday, August 28, 2017 10:32 AM  
**To:** Shmilovich, Michael (NIH/NHLBI) [E] <[michael.shmilovich@nih.gov](mailto:michael.shmilovich@nih.gov)>; Bordine, Roger (NIH/OD) [E] <[roger.bordine@nih.gov](mailto:roger.bordine@nih.gov)>; Deutch, Alan (NIH/NHLBI) [E] <[deutcha@nhlbi.nih.gov](mailto:deutcha@nhlbi.nih.gov)>  
**Cc:** [Deborah.Kassilke@nih.gov](mailto:Deborah.Kassilke@nih.gov); Rohrbaugh, Mark (NIH/OD) [E] <[rohrbaum@od.nih.gov](mailto:rohrbaum@od.nih.gov)>; Thomas, Gina (NIH/OD) [E] <[gthomas@od.nih.gov](mailto:gthomas@od.nih.gov)>  
**Subject:** RE: KEI Request FOIA Request Re: CRADAs Executed 2010-2017

Good Morning,

I have not yet responded, and I have cc'd Gina Thomas at OTT FOIA as well. She is handling two FOIA request cases from KEI about CRADA lists and tech transfer records.

Let me know when is best to talk with all of you so we can figure out the best way to respond soon.

Thanks.

**Roger Bordine**  
Program Assistant  
Freedom of Information Office  
National Institutes of Health  
Building 31, Room 5B35  
31 Center Drive

REL0000024065

Bethesda, MD 20892

Phone: 301-496-5633

Fax: 301-402-4541

[Roger.bordine@nih.gov](mailto:Roger.bordine@nih.gov)



National Institutes of Health  
*Turning Discovery Into Health*

**From:** Shmilovich, Michael (NIH/NHLBI) [E]

**Sent:** Monday, August 28, 2017 9:52 AM

**To:** Bordine, Roger (NIH/OD) [E] <[roger.bordine@nih.gov](mailto:roger.bordine@nih.gov)>; Deutch, Alan (NIH/NHLBI) [E] <[deutch@nhlbi.nih.gov](mailto:deutch@nhlbi.nih.gov)>

**Cc:** NIH FOIA <[nihfoia@od.nih.gov](mailto:nihfoia@od.nih.gov)>; [Deborah.Kassilke@nih.gov](mailto:Deborah.Kassilke@nih.gov); Rohrbaugh, Mark (NIH/OD) [E] <[rohrbaum@od.nih.gov](mailto:rohrbaum@od.nih.gov)>

**Subject:** RE: KEI Request FOIA Request Re: CRADAs Executed 2010-2017

Roger -

b5

b5

Thank you!!

**Michael A. Shmilovich, Esq., CLP**



National Heart, Lung,  
and Blood Institute

Office of Technology Transfer and Development

31 Center Drive Room 4A29, MSC2479

Bethesda, MD 20892-2479

o. 301.435.5019

[shmilovm@mail.nih.gov](mailto:shmilovm@mail.nih.gov)

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**From:** [jamespackardlove@gmail.com](mailto:jamespackardlove@gmail.com) [mailto:[jamespackardlove@gmail.com](mailto:jamespackardlove@gmail.com)] **On Behalf Of** Jamie Love

**Sent:** Monday, August 28, 2017 08:28

**To:** Bordine, Roger (NIH/OD) [E] <[roger.bordine@nih.gov](mailto:roger.bordine@nih.gov)>

**Cc:** Claire Cassedy <[claire.cassedy@keionline.org](mailto:claire.cassedy@keionline.org)>; NIH FOIA <[nihfoia@od.nih.gov](mailto:nihfoia@od.nih.gov)>; [Deborah.Kassilke@nih.gov](mailto:Deborah.Kassilke@nih.gov); Shmilovich, Michael (NIH/NHLBI) [E] <[michael.shmilovich@nih.gov](mailto:michael.shmilovich@nih.gov)>; Rohrbaugh, Mark (NIH/OD) [E] <[rohrbaum@od.nih.gov](mailto:rohrbaum@od.nih.gov)>

**Subject:** KEI Request FOIA Request Re: CRADAs Executed 2010-2017

Dear Roger Bordine,

REL0000024065



I am attaching some correspondence I have had the NIH over the issue of CRADAs. When we respond to an NIH request for comments on an exclusive license, we often ask for the CRADA, if any, associated with the license. For example, recently we requested the CRADA associated with the miRecule CRADA, which involves a recent former NIH employee. Typically, as in the case of MiRecule, the NIH licensing officials refuses to give us a copy of the CRADA, claiming it is confidential. We both know that the CRADA document is in fact subject to FOIA, but FOIA takes a long time, can will not be processed before the comment period closes.

When we asked the Office of the Director for a list of all CRADA agreements earlier this year, we were told that the NIH would not provide such a list, because the information was in a computer database and the NIH was not required to create the list from the database under FOIA. We noted at the time that this would force us to FOIA all of the CRADAs, which we thought would be a waste of everyone's time, an opinion that you seemed to share.

Why doesn't the NIH do what some other federal agencies do and list the CRADAs, all of them, on the NIH web page, to enhance the transparency of the licensing and technology transfer operations?

In any event, please decide if the NIH wants to provide a list of the CRADAs or not, and if we have to sue to get copies if you won't in fact provide such a list.

The NIH knows full well the Congress, the press, academic researchers, taxpayer and patient advocacy groups all want to have more transparency of NIH technology transfer activities. The continual stonewalling of legitimate requests for public documents is inappropriate for an agency like the NIH that manages billions of taxpayer dollars to address important health issues, and where the pricing of NIH funded products is a major concern.

In the meantime, please provide KEI with a copy of the miRecule CRADA, and the list of the CRADAs, asap.

James Love  
Knowledge Ecology International

Attached are portions of some previous correspondence with the NIH.

----- Forwarded message -----

From: **NIH FOIA** <[nihfoia@od.nih.gov](mailto:.nihfoia@od.nih.gov)>

Date: Wed, Aug 16, 2017 at 5:15 PM

Subject: RE: Request FOIA Request Re: CRADAs Executed 2010-2017

To: Claire Cassedy <[claire.cassedy@keionline.org](mailto:claire.cassedy@keionline.org)>

Cc: NIH FOIA <[nihfoia@od.nih.gov](mailto:.nihfoia@od.nih.gov)>

Good Afternoon,

Thank you for your NIH FOIA request.

Upon reading your request, it appears as though you are asking for all CRADAs from the NIH between 2010-2017, and as it stands, that aspect of your request is too broad and would involve searching records from of all of the 27 institutes and centers at the NIH.

Searching for this many records, and the review efforts afterwards, would put an undue burden on Federal Government resources, as stipulated in the FOIA, and as such, requires you to narrow the scope of your request.

It is estimated that, within your requested timeframe, there would be hundreds of CRADAs across the NIH's institutes, and if you would like to submit a new/revised request detailing a smaller number of specifically named/individual CRADAs, you are more than welcome to request those records. If not, and you would rather request just a list of CRADAs and not the CRADA records themselves, you may do that instead.

Please let us know if you would like to withdraw this initial request in favor of submitting a new request for clarified/named records.

Thank you, and please let us know if you have any questions.

**Roger Bordine**

Program Assistant

Freedom of Information Office

National Institutes of Health

Building 31, Room 5B35

31 Center Drive

Bethesda, MD 20892

Phone: 301-496-5633

Fax: 301-402-4541

Roger.bordine@nih.gov



----- Forwarded message -----

From: **James Love** <[jamespackardlove@gmail.com](mailto:jamespackardlove@gmail.com)>

Date: Thu, Jan 19, 2017 at 7:34 PM

Subject: Re: Your requests for information from NIH OTT

To: "Rohrbaugh, Mark (NIH/OD) [E]" <[RohrBauM@od.nih.gov](mailto:RohrBauM@od.nih.gov)>

Cc: "Kassilke, Deborah (NIH/OD) [E]" <[deborah.kassilke@nih.gov](mailto:deborah.kassilke@nih.gov)>, "claire.cassedy@keionline.org" <[claire.cassedy@keionline.org](mailto:claire.cassedy@keionline.org)>

We can't FOIA a database or require records be generated under FOIA. We can FOIA every CRADA, which is what we are going to be forced to do.

But if we knew what records were in the database, a query might save everyone a lot of time.

On Fri, Jan 20, 2017 at 1:07 AM, Rohrbaugh, Mark (NIH/OD) [E] <[RohrBauM@od.nih.gov](mailto:RohrBauM@od.nih.gov)> wrote:

There is no "list" but we do have a database with CRADA and license information.

**From:** James Love [mailto:[jamespackardlove@gmail.com](mailto:jamespackardlove@gmail.com)]

**Sent:** Thursday, January 19, 2017 7:01 PM

**To:** Kassilke, Deborah (NIH/OD) [E] <[deborah.kassilke@nih.gov](mailto:deborah.kassilke@nih.gov)>

**Cc:** [claire.cassedy@keionline.org](mailto:claire.cassedy@keionline.org); Rohrbaugh, Mark (NIH/OD) [E] <[RohrBauM@OD.NIH.GOV](mailto:RohrBauM@OD.NIH.GOV)>

**Subject:** Re: Your requests for information from NIH OTT

These are the types of data that make it hard to believe you don't have registry or list of the CRADAs.

<https://www.ott.nih.gov/tt-metrics/crada-metrics>

On Fri, Jan 20, 2017 at 12:56 AM, James Love <[jamespackardlove@gmail.com](mailto:jamespackardlove@gmail.com)> wrote:

Thank you.

We do note that the NIH is able to report the total number of CRADAs in any given year, and also that that number is quite a bit smaller than the number of CRADAs noticed in the federal register.

For number of CRADAs, <https://www.ott.nih.gov/ott-statistics>

We are mostly interested in the Standard CRADAs.

We thought if the NIH could provide a count of the number of CRADAs, they must have a registry or list or database that lists the CRADAs, with the name of the CRADA partner and the purpose of the CRADA.

We were surprised when we were told that no such lists exist.

The CRADAs mentioned in the annual reports do not seem inclusive of all CRADAs in a given year.

For example:

In FY15, NIH Institutes executed 5,826 of these collaboration and transfer agreements, including 101 new Cooperative Research and Development Agreements (CRADAs).

I don't think there are 101 CRADAs listed in the annual report, or even the 73 for Standard CRADAs.

So, while the Annual report is useful and interesting, we still don't know who is getting the standard CRADAs.

Also, does the NIH issue exclusive licenses under the CRADAs that are not noticed in the federal register? We were told that the NIH practice was to not provide public notice and comment on all CRADAs and that public notice and comment is not available for all exclusive licenses from CRADAs.

Jamie

On Fri, Jan 20, 2017 at 12:26 AM, Kassilke, Deborah (NIH/OD) [E] <[deborah.kassilke@nih.gov](mailto:deborah.kassilke@nih.gov)> wrote:

Mr. Love –

Recently your office contact me and two other employees in my office with questions concerning royalty payments, the use of the Federal Registry in tracking NIH CRADAs, and a request for information on the process by which the NIH enters into a CRADA with an industry collaborator. I am aware that Mark Rohrbaugh (cc'd) spoke directly with Claire Cassidy to discuss many of the CRADA related process components including the use of

Federal Register notices and how IP is addressed in a CRADA. If you still have questions regarding the use of CRADAs at NIH, we can certainly schedule another call with you.

I confirmed that the NIH FOIA office is still working on a FOIA request for you concerning royalty payment information. They apologize for the delay, but the FOIA office is short staffed at this time and they are working diligently to hire and train new staff. We just last week brought in an Acting Director for the FOIA office, Katherine Uhl, who is on detail to us from the FDA. She is working diligently to keep the plates spinning and asked that I relay to you they are working on the request. Ms. Uhl invites you to contact her office for a status of your FOIA request if you so desire; that number is 301-496-5633.

I hope that you are aware that our annual reports and statistics can be found on our website in the "MEDIA Room" tab; they may be helpful to you.

Please let me know if you would like another call scheduled with Mark and me; we will gladly set something up.

Deb

*Deborah Kassilke*

*Director, Office of Technology Transfer*

*National Institutes of Health*

*6011 Executive Boulevard, Suite 325*

*Rockville, MD 20852*

*E-Mail:*

*Deborah.Kassilke@nih.gov*

*Phone: 301-435-5294*

*Cell:* b6



**From:** Claire Cassedy [mailto:[claire.cassedy@keionline.org](mailto:claire.cassedy@keionline.org)]

**Sent:** Tuesday, August 15, 2017 11:38 AM

REL0000024065

To: NIH FOIA <nihfoia@od.nih.gov>

Subject: Request FOIA Request Re: CRADAs Executed 2010-2017

Dear FOIA Officer,

Please find attached a Freedom of Information Act request from Knowledge Ecology International regarding Cooperative Research and Development Agreements executed by the NIH from 2010 to 2017. Thank you in advance for your attention to this request.

Sincerely,

Claire Cassedy

----- Forwarded message -----

From: **Shmilovich, Michael (NIH/NHLBI) [E]** <michael.shmilovich@nih.gov>

Date: Fri, Aug 18, 2017 at 10:34 AM

Subject: FW: miRecule CRADA

To: "jamespackardlove@gmail.com" <jamespackardlove@gmail.com>

Cc: "Deutch, Alan (NIH/NHLBI) [E]" <deutcha@nhlbi.nih.gov>, "Bailey, Brian (NIH/NHLBI) [E]" <bbailey@nhlbi.nih.gov>

Jamie – All scientific, business and financial information pertaining to the CRADA between MiRecule and NIDCD other than what has already been made public by either by publication, published patent applications or other public disclosures, is strictly confidential. As such, we cannot provide you with a copy of that agreement.

Regards,

Michael A. Shmilovich, Esq., CLP

-----  
22 August 2017  
James Packard Love  
Knowledge Ecology International  
1621 Connecticut Avenue, Suite 500

REL0000024065

Washington, DC 20009

<http://keionline.org>

Work: +1.202.332.2670; Mobile: +1.202.361.3040

[james.love@keionline.org](mailto:james.love@keionline.org)

IN RE: 82 Fed. Reg. 36809 (August 7, 2017), "Prospective Grant of Exclusive Patent License: MicroRNA therapeutics for treating squamous cell carcinomas" to miRecule, Inc.

Dear Mr. Love:

....

Dr. Saleh will have direct participation in the research under his company's Cooperative Research and Development Agreement (CRADA) with the National Institute on Deafness and Other Communication Disorders (NIDCD) in order to advance the technology since a positive research outcome under the CRADA is one step closer to the development of a successful therapeutic to at least one squamous cell carcinoma. With respect to your request for various reports including CRADA documents, it is not consistent with our mission to create reports requested by the public and the proprietary content of the agreement governing the CRADA between the NIDCD is strictly confidential. In summary, the CRADA research plan sets forth a joint effort between miRecule and NIDCD to develop chemically modified mimic or mimetic microRNAs that are stable and less susceptible to nuclease degradation than previously identified microRNAs and that serve as therapeutics for cancer when delivered using tumor targeted nanoparticles. The CRADA will test these microRNAs in animal cancer models to evaluate their efficacy and the pharmaceutical properties of candidate formulations.

If your organization requests more documentation, such requests should be filed under the Freedom of Information Act. The webpage for the NIH FOIA Office provides more information on filing requests <http://www.nih.gov/institutes-nih/nih-office-director/office-communications-public-liaison/freedominformation-act-office/submitting-foia-requests>.

Michael A. Shmilovich, Esq., CLP

--

James Love. Knowledge Ecology International

<http://www.keionline.org/donate.html>

KEI DC tel: +1.202.332.2670, US Mobile: +1.202.361.3040, Geneva Mobile: +41.76.413.6584, [twitter.com/jamie\\_love](https://twitter.com/jamie_love)